National Institute
of
Allergy and Infectious
Diseases

Annual Report of Intramural Activities

October 1, 1991-September 30, 1992

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

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of
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1992 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00013-29 LIP
00020-17 ODIR
00027-25 LMM
00030-24 LI
00035-17 LI
00036-27 LI
00045-24 LCI
00047-23 LHD
00057-19 LCI
00058-18 LCI
00072-21 LPVD
00074-20 LPVD
00085-15 LPVD
00086-15 LPVD
00094-33 LPD
00097-34 LPD
00098-36 LPD
00099-22 LPD
00102-18 LPD
00108-21 LMR
00123-26 LVD
00126-19 LVD
00134-30 LI
00143-23 LIG
00144-28 LIG
00145-25 LIG
00154-17 LCI
00155-17 LHD
00161-15 LPD
00162-16 LPD
00166-15 LIG
00168-15 LIG
00169-15 BRB
00170-15 LIG
00171-15 LIG
00172-14 BRB
00180-14 LIG
00190-14 LMM
00193-13 LMSF
00197-13 LPD
00208-12 LMR
00210-12 LIR
00213-12 LIR
00216-12 LICP
00218-11 LMM
00224-11 LI
00226-11 LI

Z01-AI
0240-11 LMR
00241-11 LMR
00246-10 LPD
00248-11 LMR
00249-11 LCI
00251-11 LPD
00253-11 LPD
00255-11 LPD
00256-11 LPD
00257-11 LPD
00260-11 LPVD
00262-11 LPVD
00263-11 LPVD
00265-11 LPVD
00266-11 LPVD
00279-11 LCI
00284-11 LIP
00286-11 LIP
00294-11 LVD
00295-11 LVD
00296-11 LVD
00297-11 LVD
00298-11 LVD
00300-11 LMM
00301-11 LMM
00304-11 LMM
00306-11 LVD
00307-11 LVD
00311-11 LID
00314-11 LID
00323-11 LID
00324-11 LID
00325-11 LID
00326-11 LID
00327-11 LID
00333-11 LID
00339-11 LID
00340-11 LID
00341-11 LID
00342-11 LID
00343-11 LID
00345-11 LID
00347-10 LPD
00349-09 LI
00350-10 LPD
00352-10 BRB
00354-10 LCI

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1992 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00356-10 LCI
00358-10 LIR
00361-10 LIR
00368-10 LID
00370-10 LID
00372-10 LID
00389-08 LIG
00390-09 LIR
00394-09 LI
00403-09 LI
00415-08 LMM
00416-09 LVD
00418-09 LPVD
00425-08 LI
00427-08 LI
00429-08 LCI
00430-08 LCI
00431-08 LIR
00432-08 LCI
00439-08 LPD
00441-08 LICP
00445-08 LVD
00446-08 LID
00459-08 LID
00465-07 LIP
00467-07 LMM
00468-07 LPVD
00469-07 LCI
00470-07 LCI
00480-07 LVP
00481-07 LHD
00483-07 LMR
00484-06 LIP
00485-06 LCMI
00486-06 LCMI
00487-06 LPD
00488-06 LVP
00492-06 LVP
00493-06 LI
00494-06 LPD
00495-06 LCI
00496-06 LID
00498-06 LID
00500-06 LID
00507-06 LID
00512-05 LPD

Z01-AI
00513-05 LCI
00514-05 LCI
00516-05 LMSF
00518-05 LICP
00519-05 LICP
00520-05 LI
00521-05 LHD
00522-05 BRB
00523-05 BRB
00525-05 LIG
00527-05 LMM
00528-05 LMM
00529-05 LCMI
00530-05 LID
00531-05 LID
00533-05 LID
00534-05 LID
00537-05 LIR
00538-05 LVD
00539-05 LVD
00540-05 LVD
00541-05 LVD
00542-05 LVD
00543-05 BRB
00544-04 LIP
00545-04 LI
00547-04 LMM
00548-04 LCI
00549-04 LMSF
00552-04 LVP
00553-04 LVP
00554-04 LVP
00562-04 LIR
00564-04 LVD
00565-03 LI
00566-03 LI
00567-02 LICP
00568-03 LMSF
00569-03 LID
00570-03 LID
00571-03 LID
00572-03 LID
00573-03 LID
00574-03 LID
00576-03 LID
00578-03 LIP

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1992 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI
00579-03 LPD
00580-03 LPVD
00581-03 LCMI
00585-03 LIR
00586-03 LIR
00587-03 LIR
00589-03 LCI
00590-03 LCI
00591-03 LCI
00592-03 LCI
00593-03 LCI
00594-03 ODIR
00595-02 LCI
00596-02 LID
00597-02 LID
00598-02 LID
00600-02 LID
00601-02 LID
00602-02 LID
00603-02 LID
00604-02 LID
00605-02 LID
00607-02 LCI
00608-02 LMSF
00609-02 LMSF
00611-02 LPVD
00612-02 LICP
00613-02 LCMI
00614-02 LHD
00615-02 LHD
00616-02 LID
00617-02 LCI
00619-02 LVD
00620-02 LVD
00621-02 LCI
00622-01 LI
00623-01 LI
00624-01 LI
00625-01 LI
00626-01 LI
00627-01 LI
00628-01 LI
00629-01 LMR
00630-01 LI
00631-01 LICP
00632-01 LMSF
00633-01 LMSF

Z01-AI
00634-01 LIR
00635-01 LIR
00636-01 LIR
00637-01 LID
00638-01 LID
00638-01 LID
00639-01 LID
00640-01 LID
00641-01 LID
00642-01 LID
00643-01 LID
00644-01 LHD
00645-01 LHD
00646-01 LHD
00647-01 LHD
00648-01 LHD
00649-01 LHD
00650-01 LCI
00651-01 LCI
00652-01 LVD
00653-01 LVD
00654-01 LCI
00655-01 LCI
00656-01 LCI
00657-01 LCI
00658-01 LVD
00659-01 LVD0
00660-01 LVD
00661-01 LVD
00662-01 ODIR
00663-01 ODIR
00664-01 ODIR
00665-01 ODIR
00666-01 ODIR
00667-01 ODIR
00668-01 ODIR

TERMINATED PROJECTS
Z01 AI

00043-27 LCI
00048-21 LCI
00182-12 ODIR
00183-12 ODIR
00244-09 LPD
00271-09 LCI
00275-10 LCI
00278-09 LCI
00346-10 LID
00353-09 LMM
00397-07 LCI
00437-06 LMM
00438-06 LMM
00476-06 LID
00477-06 LID
00478-05 LID
00499-04 LID
00501-05 LID
00502-05 LID
00524-05 LPVD
00532-03 LID
00546-02 LMM
00550-04 LPVD
00551-04 LPVD
00555-02 LID
00556-03 LID
00557-03 LID
00558-03 LID
00575-02 LID
00577-01 LID
00582-02 LID
00583-02 LID
00584-01 LID
00588-02 LMM
00599-01 LID
00606-01 LID
00610-02 LPVD
00618-01 LCI

INACTIVE PROJECTS

Z01 AI
00135-16 LIP
00138-15 LIP
00205-09 LIP
00308-10 LID
00451-07 LID
00476-05 LID
00477-05 LID
00556-02 LID
00575-01 LID

TRANSFERRED PROJECTS

Z01-AI
00047-21 LPD
(FROM LCI)
00108-21 LMR
(FROM LPD)
00155-16 LHD
(FROM LCI)
00208-12 LMR
(FROM LPD)
00240-11 LMR
(FROM LPD)
00241-11 LMR
(FROM LPD)
00248-11 LMR
(FROM LPD)
00481-06 LHD
(FROM LCI)
00483-07 LMR
(FROM LPD)
00521-04 LHD
(FROM LCI)
00595-02 LCI
(FROM BRB)

OFFICE OF THE DIRECTOR, DIVISION OF INTRAMURAL RESEARCH, NIAID
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SUMMARY OF PROGRAM
Laboratory and Clinical Research, NIAID
October 1, 1991 - September 30, 1992

The Division of Intramural Research (DIR), NIAID consists of 16 laboratories, a Biological Resources Branch, Administrative Management Branch and an Animal Care Branch. Four laboratories are located in Hamilton, Montana at the Rocky Mountain Laboratories (RML). The remaining facilities are concentrated at the NIH Bethesda Campus with auxiliary facilities the Frederick Cancer Research and Development Center, Frederick, and Rockville, Maryland. The Office of the Director, DIR, (also called the Office of the Scientific Director, NIAID) is responsible for the management of the Division of Intramural Research as well as advising the Director, NIAID on scientific issues. In FY '92 the DIR, NIAID had an allocation of $119,054. The allocation of the budget was as follows: 33% for salaries, 41% for laboratory support, 9.02% for the management fund and 17% for patient costs. In FY '92 there were over 1015 persons working within the DIR, NIAID with 91 tenured scientists, 542 nontenured scientists (including guest workers, special volunteers and summer students) and about 382 nonscientists.

Numerous outstanding accomplishments were made by DIR, NIAID scientists in FY '92. Some of the many highlights were as follows:

AIDS

- Scientists developed two diagnostic assays to detect HIV infection in children born to HIV-infected mothers. One measures viral antigen and the other measures IgA immunoglobulin in infants, allowing doctors to identify infected children within the first few months of life, and thereby enabling technicians to institute antiviral therapy.

- Using mice experiments, researchers have identified several regions in the gp120 and pol proteins of HIV-1 that bind to MHC class I or class II molecules and shown that these regions also are the targets for T cell recognition by human T cells. Without the formation and recognition of the peptide-MHC complexes, the T cell immune system is blind to the HIV-1 and cannot eliminate infected cells or make antibodies that would reduce the spread of the infection in the body. Understanding the recognition of viral proteins by T cells is essential to the effective design of prophylactic and therapeutic **vaccine strategies** for the control of AIDS.

- Using an immunodeficient mouse into which normal human fetal thymus tissue is implanted, scientists are studying how the thymus is involved in HIV infection. Scientist are finding that immature T cells in the thymus can be infected with HIV. Also the cells which provide the nurturing growth signals to developing T cells appear to be affected by HIV infection. This disruption of thymus gland function, then, may contribute to the depletion of CD4+ T cells seen in patients and the dysfunctioning of the immune system of which T cells are so great a part. By understanding how HIV interrupts the normal growth and development of the immune cells in the bone marrow and thymus, scientists can gain insight int the development of therapies to prevent or counteract this immune destruction. Studies evaluating the alteration of immune function through HIV effects on bone marrow, thymus and cytokine signalling will provide knowledge about the changing immune system which will affect the design of specific vaccines for maintaining good anti-HIV immune function.

o By exchanging pieces of viral nucleic acid between clones capable of infecting different cell types, scientists have found that a small segment of the viral coat protein of the HIV has a major influence on determining the ability of a particular HIV strain to infect a particular cell type. The results may help explain why different AIDS patients have different clinical symptoms and may also have some bearing on selection of appropriate HIV strains to use in **potential HIV vaccines**.

o Scientists have learned that the cytokines IL-10, IL-4 and TGF-beta inhibit the killing of Toxoplasma gondii parasites by macrophages in infected laboratory animals. They also have found that CD8+ cells appear to play a major role in resistance against the parasite. These discoveries will be useful in designing vaccines and immunotherapies for preventing toxoplasmosis in AIDS patients and other high risk individuals.

o Scientists have found a **vaccine** which protects mice against cryptococcosis and gives an immune response in normal volunteers. They plan to test HIV infected volunteers to see if they respond to the vaccine. They also plan to test the ability of protecting mice using antibodies made in vaccinated humans. Scientists also plan to improve the vaccine by making one against the protein in the fungal capsule.

o Scientists who are studying ways in which HIV infects cells in the bone marrow, leading to the anemia and low white cell counts in patients, have found that a very early bone marrow cell which gives rise to all of the other cells in the blood stream is itself infected with HIV in some patients. This may result in the death of these early progenitor cells and contribute to the anemias which can develop in patients. By understanding how HIV interrupts the normal growth and development of the immune cells in the bone marrow and thymus, scientists can gain insight into the development of therapies to prevent or counteract this immune destruction. Studies evaluating the alteration of immune function through HIV effects on bone marrow, thymus and cytokine signalling will provide knowledge about the changing immune system which will affect the design of specific vaccines for maintaining good anti-HIV immune function.

o Scientists identified risk factors of both heterosexual and perinatal transmission of HIV. They have found that HIV infection is rapidly increasing among heterosexuals, with infection rates of 5-10% in pregnant women to as high as 40% among female prostitutes. Among patients attending STD clinics in Baltimore, HIV infection increased from 0.2%

to 5.5%, and the infection rate is now equal among both men and women. The studies have shown a strong association between acquisition of HIV infection among heterosexuals who have either past or active STDs and who use condoms infrequently. The studies also have clearly demonstrated that individuals at risk for STDs such as syphilis, herpes, chlamydia, and gonorrhea are at a high risk for HIV infection. Effective control of these STDs with increased counseling and education of high-risk individuals should help control the spread of HIV, scientists concluded.

- Scientists identified an effective target for antiviral agents against the HIV, using mutations in the reverse transcriptase coding region. They identified two mutations that when introduced into the virus, destroyed its capability to infect host cells. A viral sequence containing this mutation which prevents virus production, in addition to two mutations in other regions of the viral genome, has been introduced into cells that support HIV growth to see if a normal virus infecting such a cell will produce viruses which contain the mutated RT enzyme. If it does, the newly formed virus would be non-infectious.

- Researchers identified a ribozyme (catalytic RNA) that suppresses HIV-1 replication.

ASTHMA/ALLERGY

- Researchers classified the patterns of disease found in mastocytosis, which will allow for better management of the disease. In addition, they identified tests that can be used to diagnose mastocytosis. These tests can also be applied to other diseases that involve mast cells, such as anaphylaxis. Also, they determined that a number of drugs that stabilize mast cells are useful in mastocytosis and may be of possible benefit in the treatment of allergic and other immunologic disorders.

- Allergens bind to IgE antibodies, which in turn are attached to receptors on various cells in the immune system. The binding of the allergen causes the cells to release the mediators of allergic reactions. Researchers have characterized the IgE receptor, which is the key molecule for the cell activation. This should help in the design of new molecules that could interfere with the binding of IgE to its receptor.

CHRONIC AND PERSISTENT INFECTION

- Scientists have detected expression of certain VZV gene products in certain nervous system tissues (trigeminal ganglia), indicating that these genes are expressed even when the virus is in its dormant state in the human body. Scientists are analyzing the structure of these and other VZV gene products and have shown that these genes can upregulate expression in other VZV genes. They also have constructed cell lines that express several of these VZV regulatory proteins and have found that one of the VZV genes can functionally substitute for the homologous protein of another herpesvirus. Identifying and determining the function of VZV genes that are important for infection with the virus (chickenpox) and for reactivation of the virus from dormancy (shingles) will provide insight into therapies and vaccines.

o Infection with C. trachomatis often leads to chronic inflammatory conditions that result in blindness and infertility. Using an in vitro cell culture system, scientists have shown that Interferon-gamma (IFN-gamma), an immune enhancing substance produced naturally in the body, causes persistent infections in chlamydia. In the studies, IFN-gamma inhibited the production of infectious progeny, despite the presence of chlamydiae inside the cells. Analysis of infected cells treated with IFN-gamma revealed morphological changes in chlamydiae characterized by large abnormal intracellular forms. These atypical forms were found to exhibit differential expression of chlamydial antigens. When persistently infected, the cells expressed an abundance of the chlamydial heat shock protein hsp60 and a very little of the major outer membrane protein (MOMP). If IFN-gamma has a similar effect in vivo, then persistent infections could be very important in the chronic inflammatory sequelae of chlamydial disease by functioning as the source of antigen for the stimulation of chronic inflammatory responses that lead to blindness and infertility.

DIGESTIVE/ENTERIC DISEASES

o Investigators have initiated a new approach to the identification of rotavirus RNA sequences and proteins involved in viral gene expression, replication, and virion morphogenesis. They developed a simplified system in which cDNA is used to encode synthetic analogs of a rotavirus RNA. The cDNA-encoded chimeric RNAs could be rendered biologically active when introduced into tissue culture cells and complemented by proteins supplied by superinfecting rotavirus. This provides a system that should facilitate the investigation of cis-acting RNA sequences involved in RNA transcription, replication, and nucleocapsidation. This approach will be developed further to provide a system for identifying and characterizing rotavirus proteins involved in key activities. Ultimately, this approach might also be used to "rescue" synthetic cDNA-encoded rotavirus gene segments by incorporating them into infectious virus. The capability of introducing synthetic RNAs into infectious viruses represents an important line of investigation with regard to the development of live attenuated rotavirus strains for vaccine purposes.

- Inflammatory bowel disease (IBD) is a relatively common disorder that leads to severe gastrointestinal symptoms, poor growth in children, and tumors. In people with IBD, researchers discovered a significant decrease in the quantity of a type of T cell antigen receptor designated TCR-Vβ_2. This suggests that a particular antigen is involved in causing IBD.

GENE TRANSFER, MAPPING, AND GENETIC DISEASES

- Scientists demonstrated in vitro that retroviral transduction can result in functional correction of one genetic form of CGD in which cells fail to synthesize a protein called $p47^{phox}$. Scientists developed two model systems to demonstrate the feasibility of genetic correction of $p47^{phox}$ deficient CGD. The first system used a cell line derived from lymphocytes of CGD patients (EBV-BL cell lines). EBV-BL from normal individuals produce small amounts of hydrogen peroxide when stimulated, while those derived from $p47^{phox}$ deficient CGD patients do not produce hydrogen peroxide. Retrovirus containing $p47^{phox}$ genetically corrected the ability of CGD EBV-BL to produce small amounts of $p47^{phox}$ protein and in related studies, resulted in 15% restoration of hydrogen producing capacity. In the second model system, marrow-like blood precursor cells harvested from the peripheral blood could be induced to grow and divide in culture. Unlike normal blood cells derived from these precursors, those from $p47^{phox}$ deficient CGD patients could not produce hydrogen peroxide. The retrovirus-$p47^{phox}$ construct inserted into these precursors from CGD patients resulted in correction of superoxide production in 7% of resultant phagocytes. Scientists say it may be possible to use this procedure to do in vivo genetic therapy of this form of CGD.

- Researchers have developed methods for identifying and purifying multipotent (stem) cells, which are extremely rare components of the blood-forming organs and which are able to differentiate to form all of the diverse types of cells found in normal blood. Since these cells survive in cell cultures, it might be possible to study blood development *in vitro*, thereby reducing the need for experimental animals. In addition, genetic disorders could potentially be treated with transplants of genetically engineered stem cells.

- Production of a high density mouse linkage map is one of the major goals of the effort to characterize the mammalian genome, since mouse is an important model. Scientists reported new map locations in the past year for 20-30 genes, including the mouse homolog of the cystic fibrosis gene, genes for heat shock proteins, enzymes, proto-oncogenes, and genes with various tissue restricted patterns of expression. One of these genes, expressed only in melanocytes, maps near the silver coat color mutation, and a brain-specific gene maps near the coloboma neurological mutation and is deleted in these mice. The two genes are therefore under further investigation as molecular candidates for these inherited disorders.

- Investigators have identified a possible retroviral resistance gene in mice. They inserted this gene into fertilized mouse embryos, thereby producing "transgenic" mice that carried the gene and were resistant to retroviral infection. The scientists will be investigating the possibility of inserting the gene into mouse cells *in vitro* and then introducing the treated cells into mice to see if they induce resistance.

IMMUNOLOGY

- Investigators have established that Class I and Class II MHC molecules are specialized for binding to antigenic peptides in different parts of the cell. They found, while Class I molecules are good at detecting antigens made by the cell such as viral proteins, Class II molecules are better at detecting antigens outside of the cells, such as toxins, or debris from dying organism. The scientists have also discovered how the MHC molecules capture peptide antigen: they use the peptide to help them fold into a shape that lets them move from inside the cell to the surface where a T cell can see the peptide-MHC complex. They also have started to identify the places inside the cell where the peptides are made and the special mechanism that help the cell join up the antigenic peptide and the MHC molecule and then move the complex to the cell surface. These studies are permitting the direct identification of the materials causing autoimmune disease and the antigens important in fighting invading microorganisms. Understanding about MHC molecule is guiding clinical studies on relationship between MHC and disease and the design of anti-autoimmunity agents and formulation of new vaccines and vaccine delivery methods.

- The pattern of T cell responses to invading microorganisms is largely determined by the type of cytokines that these T cells produce. T cells that produce IL-4 stimulate B cells to produce antibodies, whereas T cells that produce interferon-gamma induce responses that are protective against intracellular microorganisms. Scientists discovered that what determines whether a T cell is going to produce IL-4 or interferon-gamma is the presence of absence of IL-4 at the time the T cells are initially exposed to the antigen. If IL-4 is present, the T cells produce IL-4; if IL-4 is not present, the T cells produce interferon-gamma. This information will help in the design of vaccines, particular those directed against intracellular organisms.

- T cells only respond to foreign antigens that are displayed on the surface of presenting cells. The signal generated by the T cell recognition of antigen is not sufficient to activate the T cell. A costimulatory signal, delivered by the presenting cell, is also required. The signal appears to be generated when a molecule on the presenting cell, known as B7/BB-1, interacts with molecules on the T cell, which are called CD28 and CTLA-4. LCMI investigators measured the ability of T cells to proliferate in response to interleukin-4 (IL-4) and discovered that costimulation is required for T cells to become competent to respond to IL-4.

This activation process can be blocked with a solubilized form of the CTLA-4 receptor. These results suggest that it may become possible to design immunotherapeutics that interfere with the delivery of the second signal and that will be useful in treating autoimmune diseases and facilitating organ transplantation.

- Scientists developed a deeper understanding of how MHC molecules can have either of two effects on antigen presentation to T cells. They now realize that MHC molecules don't fold properly inside the cell unless peptide is attached and that this change in structure induced by peptide binding is essential for the movement of MHC molecules to the cell surface where a T cell can see them. This peptide-dependent change in structure occurs differently for the two types of MHC molecules, class I and class II, helping to explain the specialization of these two classes for binding peptides in different places inside the cell.

- Class I major histocompatibility complex (MHC) proteins are involved in alerting cytotoxic T cells to the presence of body cells that have been changed for the worse--for example, infected with a virus--and that need to be eliminated. Class I proteins combine with peptides from the foreign organism and this class I/peptide complex is transported to the surface of the infected cell, where it serves as a flag for cytotoxic T cells. Due to genetic differences, individuals vary in the binding properties of their class I molecules and therefore in their ability to resist diseases. Using class I molecules isolated from *E. coli* expression vectors, scientists have obtained enough class I protein to begin to dissect the molecular basis of peptide binding specificity for each of the many class I molecules present in the population. Knowledge of the structure of the class I/peptide complex could lead to the ability to produce large numbers of activated cytotoxic T cells *in vitro* that could be infused back into patients to protect against viral diseases.

- T lymphocytes expressing receptors encoded by the gamma and delta chain genes were discovered about 5 years ago, but a function for them has not yet been determined. Researchers injected gamma-delta T cells into severe combined immunodeficiency (SCID) mice, which lack normal T or B lymphocytes, and found that they appear to facilitate the development of the major subset of T cells that express alpha-beta receptors.

SEXUALLY TRANSMITTED DISEASES (NON-AIDS)

- Scientists identified key T and B cell antigenic determinants of the *C. trachomatis* major outer membrane protein (MOMP) the primary target antigen for the development of vaccine against chlamydial STDs. These T and B cell epitopes have been assembled together as synthetic peptide immunogen termed A8-VDIV. Peptide A8-VDIV was shown to be immunogenic in mice and primates and is capable of evoking broadly cross neutralizing antibodies effective against those *C. trachomatis* serovars that are epidemiologically important agents of chlamydial STDs. Next step in evaluation of peptide A8-VDIV as a potential vaccine for human use is to target the synthetic immunogen to evoke mucosal as well as systemic immunity and to conduct Phase I immunogenicity and toxicity trials in humans. A vaccine against *Chlamydia trachomatis* might prevent chlamydial salpingitis, a major cause of involuntary infertility among women in the United States.

- A mutant form of hepatitis B virus HBV has recently been identified. The mutant virus has an altered form of a protein that is a key component of the licensed HBV vaccine, which suggests that the vaccine may not protect people from the mutant virus. Scientists have determined that chimpanzees can be infected with the mutant virus, thereby laying the groundwork for determining whether the vaccine is effective in protecting against the mutant virus.

- Scientists found that a new antiviral drug known as FIAU dramatically reduces the blood levels of hepatitis B virus (HBV) in infected individuals. In some people, the virus was cleared from the bloodstream and the patients may be cured. The drug was effective both in AIDS patients who had a concurrent HBV infection and in individuals without AIDS. The drug has little or no side effects.

- Scientists developed and used a new PCR assay for *Chlamydia trachomatis* that has doubled the sensitivity of culture for the detection of chlamydia among sexual partnerships. This newly developed test is highly sensitive for the early detection of chlamydial infection thereby leading to earlier therapy. Also developed an assay for detection of *C. pneumoniae* will help better define the epidemiology and clinical features of *C. pneumoniae* pneumonia as well as identifying infected individuals for therapy.

- Scientists found that the gonococcal opacity protein's fourth oligopeptide loop plays a role in mediating human neutrophil responses to gonococci. The scientists also found that gonococci attach to host proteins by varying their surface composition. The ability of *Neisseria gonorrhoea* to mediate interactions with host inflammatory cells and to adhere to host proteins by varying its surface composition are important aspects of its pathogenic potential. A better understanding of the disease process may lead to new approaches to preventing the establishment of or maintenance of infection.

TROPICAL DISEASES

- Researchers are developing a vaccine for the blood stage of the malaria parasite *Plasmodium vinckei*. A vaccine made from whole parasite is infeasible because of the cost of growing the parasite and the danger that it may be contaminated with other microorganisms. Instead, the scientists are developing a recombinant vaccine consisting of a gene or genes from the parasite inserted into the DNA of the bacterium *Salmonella*.

They are currently testing various malarial genes to see which ones will lead to protection against the parasite. To date, immunizations with the recombinant vaccine have induced immune responses in mice, but the animals were only partially protected.

The DIR has continued to emphasize Equal Employment and Affirmative Action programs this year. Our annual Introduction to Biomedical Research program for minority students from across the U.S. and Puerto Rico was again sponsored jointly with the Office of the Director, NIAID. As in previous years the program was successful in bringing students to NIH for personal interactions and to participate in a science lecture program. The ten students as well as the two others from the prior year were selected for summer research positions in NIAID laboratories; were the recipients of intense one-on-one scientific training. This year we completed our first year of the "Adopt-a-School" program in conjunction with Dunbar Senior High School in Washington, DC. DIR scientists conducted 1 - 2 hour seminars with the students at Dunbar, selected Dunbar students participated in the first phase of the Introduction to Biomedical Research program and 3 Dunbar students were given positions in NIAID laboratories. Overall, the program was felt to be a great success in introducing minority students to careers in research. Between the Introduction to Biomedical Research Program and the Adopt-a-School Program some 20 minority students were included in our summer research program, or 20/37 of the total summer program. Finally, the DIR expanded a productive collaboration with Howard University by which the two Institutions exchange patient care expertise. Within the context of this collaboration DIR physicians attended patient care rounds in general medicine and infectious diseases at Howard University in Washington, DC. Howard University, in turn, sent a fellow to NIH for a month-long inpatient rotation.

In FY '92 the Division of Intramural Research made several new appointments. Dr. Louis Miller was appointed Laboratory Chief, of the newly formed Laboratory of Malaria Research (LMR). In addition, Dr. Frank Neva, Chief of the Laboratory of Parasitic Diseases was appointed Chief of a newly constituted Center for Parasitic Diseases, one of several to be established nationwide.

The NIAID Board of Scientific Counselors under the leadership of Dr. Dennis Kasper reviewed the Laboratory of Immunogenetics (Dr. Thomas Kindt, Chief), the Laboratory of Cellular and Molecular Immunology (Dr. Ronald Schwartz, Chief) and the Laboratory of Immunopathology (Dr. Herbert Morse, Chief) on December 9, 1991. Reviews for the Laboratory of Infectious Diseases (Dr. Robert Chanock, Chief) was on May 11, 1992. These reviews were generally excellent. NIAID thanks Drs. Richard Goldsby and Howard Grey who have completed their three year tours on the NIAID Board of Scientific Counselors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00594-02 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Structure and Function of Fcγ and Fcε receptors

PRINCIPAL INVESTIGATOR
Jean Pierre Kinet, Section Chief - MAIS, OSD, NIAID
Anna Teresa Brini, Visiting Assoc.
Marie-Helene Jouvin, Visiting Assoc.
Helmut Kuster, Visiting Assoc.
Li Zhang, Visiting Assoc.
Odile Mejan, Visiting Fellow
Rosella Paolini, Visiting Fellow
Martin Adamczewski, Visiting Fellow
Kenichi Ochiai, Visiting Fellow
Gai Mi Lee, Laboratory Worker
Jami Willette-Brown, Microbiologist
Fumiyoski Takizawa, Visiting Fellow
Bernie Effertz, IRTA

COOPERATING UNITS -Laboratory of Clinical Investigation, NIAID (Rottem, Metcalfe)
-Laboratory of Experimental Immunology, NCI (O'Shea, Kennedy, Ortaldo)
-Cell Biology and Metabolism Branch, NICHD (Orloff, Klusner)-John Hopkins University

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
10.5	10	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK

This project was terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00662-01 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Characterization of the FcεRI α chain by crystallography

PRINCIPAL INVESTIGATOR

PI:	Jean-Pierre Kinet, Section Chief	MAIS, NIAID	
Others:			
	Jami W. Brown	Microbiologist	MAIS, NIAID
	Odile M. Letourneur	Visiting Associate	MAIS, NIAID

COOPERATING UNITS
-Pamela Bjorkman (California Institute of Technology)
-Bruce Grasberger (Laboratory of Chemical Physics, NIDDK)
-Steve Stahl & Joshue Kaufman (Protein Expression Laboratory, NIDDK)

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	1 summer student

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

The receptor with high affinity for IgE (FcεRI) is a tetrameric complex of an IgE binding α chain, a β chain and a dimer of γ chains. In the past we have found that the extracellular region of the α chain is solely responsible for ligand binding. In order to further characterize the receptor binding site by crystallography, we have generated various transfectants capable of secreting large amounts of truncated α chain capable of binding IgE. However this material, like the native α is heavily glycosylated. This is preventing the formation of analyzable crystals. In order to circumvent this problem, we are following two different strategies: (1) The transfection of truncated α chain in cells with a defective glycosylation machinery. (2) The mutation of the seven N- linked glycosylation sites. Finally we are also attempting to produce a similar molecule in E. coli with the hope of using the NMR techniques to charaterize the receptor binding site.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-554-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00663-01 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Low affinity IgE receptors as receptors for IgE

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

F. Takizawa	Visiting Fellow	MAIS, NIAID
M. Adamczewski	Visiting Fellow	MAIS, NIAID

COOPERATING UNITS

NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK

We have demonstrated that mouse FcγRII and FcγRIII are not isotype specific and are capable of binding IgE immune-complexes. Furthermore, the binding of IgE immune-complexes to these receptors induces cell activation.

We also have generated transfectants expressing human FcγRII and FcγRIII and we are at present studying whether our findings in the mouse system will be relevant in the human system.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00664-01 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Distribution of FcεRI in human cells

PRINCIPAL INVESTIGATOR

PI:	Jean-Pierre Kinet, Section Chief	MAIS, NIAID	
Others:			
	F. Takizawa	Visiting Fellow	MAIS, NIAID
	A. Brini	Visiting Associate	MAIS, NIAID

COOPERATING UNITS

-G. Stingl (Vienne, Austria)
-M. Capron (Lille, France)

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK

We have generated a monoclonal directed against the α chain of the human FcεRI. With this reagent we found that FcεRI is expressed on the surface of Langerhans cells in human skin and we are attempting to determine the possible role of this receptor in atopic dermatitis.
We are also looking actively at the possibility that FcεRI might be expressed in other cells such as eosinophils, macrophages, NK cells etc...

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00665-01 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
The role of the FcεRI receptor subunits in signal transduction

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

M. Adamczewski	Visiting Fellow	MAIS, NIAID
M. H. Jouvin	Visiting Associate	MAIS, NIAID
R. Paolini	Visiting Fellow	MAIS, NIAID
F. Takizawa	Visiting Fellow	MAIS, NIAID
A. Valle	Special Volunteer	MAIS, NIAID

COOPERATING UNITS

NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.4	3.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK

The high affinity IgE receptor belongs to a class of receptors which lack intrinsic kinase activity, but activates non-receptor tyrosine and serine / threonine kinases. Receptor engagement triggers a chain of signalling events leading from protein phosphorylation to activation of phosphotidylinositol-specific phospholipase C, an increase in intracellular calcium levels, and ultimately the activation of more specialized functions. We have investigated the role of the β and γ chains in signal transduction. We found (1) that receptor engagement induces immediate phosphorylation of β (tyrosine and serine) and of γ (tyrosine and threonine); (2) that this phosphorylation is specific to activated receptors and is reversible upon receptor disengagement; (3) that the γ chains are critical for signal transduction but that the β chain does not seem necessary; (4) that phosphorylation / dephosphorylation is a coupling / uncoupling mechanism; (5) that the receptor activates two distinct phosphorylation pathways, one cell-specific and the other not. We are at present trying to identify the role of β and are studying further the role of receptor phosphorylation in cell activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00666-01 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
The role of the molecules interacting with FcεRI in signal transduction

PRINCIPAL INVESTIGATOR
PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID
Others:

M. Adamczewski	Visiting Fellow	MAIS, NIAID
B. Cuenod	Special Volunteer	MAIS, NIAID
L. Chung	IRTA	MAIS, NIAID
R. Paolini	Visiting Associate	MAIS, NIAID
A. Valle	Special Volunteer	MAIS, NIAID
A. Brini	Visiting Associate	
R. Numerof	IRTA	MAIS, NIAID

COOPERATING UNITS

NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.9	4.9	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

We found recently that the FcεRI receptor becomes associated with other molecules upon receptor engagement. We are attempting to characterize these molecules and to raise new monoclonal antibodies against them. We are also attempting to clone these molecules with various methods. Finally we are trying to better define the set of kinases and phosphatases involved in receptor signalling and to analyze their relative importance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00667-01 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Regulation of the expression of the human genes encoding FcεRI α and γ chains

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

A. Brini	Visiting Associate	MAIS, NIAID
F. Takizawa	Visiting Fellow	MAIS, NIAID
G. M. Lee	Laboratory Worker	MAIS, NIAID

COOPERATING UNITS

NONE

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

The γ chains of the high affinity IgE receptor are also a subunit of other receptors. They are part of the low affinity IgE receptor in NK cells and macrophages and part of the T cell antigen receptor in some subsets of T cells. We have isolated and analysed the regulatory regions of the γ chain gene and found that two major elements containing Alu sequences control the expression of this gene. One of these elements bind similar nuclear factors in basophils and T cells, while the other element bind to nuclear factors which are different in basophils and T cells.
We also have isolated the gene for the human α chain and we are in the process of characterizing the regulatory elements of this gene. In addition, we are studying the tissue distribution of expression of the α gene and the possible factors involved in the regulation of the α gene in various types of cells.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00668-01 ODIR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Production of IgE receptor deficient mice by homologous recombination

PRINCIPAL INVESTIGATOR

PI:	Jean-Pierre Kinet, Section Chief	MAIS, NIAID	
Others:			
	S. Germana	Microbiologist	MAIS, NIAID
	D. Dombrowicz	Special Volunteer	MAIS, NIAID
	J. Divic	Visiting Fellow	MAIS, NIAID

COOPERATING UNITS
Beverly Köller (The University of North Carolina)

LAB/BRANCH
Office of Scientific Director (OSD)

SECTION
Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION
NIAID, Twinbrook II Facility 12441 Parklawn Drive, Rockville, Maryland 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

We are attempting to generate mice lacking functional high affinity IgE receptor genes. These mice will help us to charaterize the physiological function of the receptor and to further study the role of the receptor in allergy.

We have cloned the alpha and beta chain genes from embryonic stem cells and have generated constructs which will be used to target the relevant genes by homologous recombination. When this is accomplished, we will attempt to produce mice in which the alpha or the beta have been knocked-out. If successful, we will be able to generate homozygotic mice in which both alleles will lack the functional genes encoding the alpha or beta gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00020-17

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies with PolyICLC

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Hilton Levy, Ph.D., Special Assistant to the Scientific Director, NIAID

COOPERATING UNITS *(if any)*

Dr. Andre Salazar Walter Reed; Dr. Meir Kende, USAMRIID; Dr. Tom Balkan, WRAIR; Dr. J. Morales, Presbyterian Hospital, Puerto Rico; Dr. Owen Morgan, U. of West Indies, Jamacia; Dr. B. Sherokman, Bethesda Naval Hospital; Dr. R. Meheshwari, USUHS.

LAB/BRANCH

Office of the Scientific Director (OSD)

SECTION

INSTITUTE AND LOCATION

NIAID, NIH - Fort Detrick, Frederick, Maryland 21702

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	1.0	.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PolyICLC, the immune enhancing and interferon inducing drug developed by these laboratories, is being tried in 4 clinical situations. 1) In chronic progressive Multiple Sclerosis, 29 patients have been entered into the study, 14 passed the time stipulated in the protocol, 15 remain on study. Almost all appear to have stabilized disease, and are not worsening, in addition, MRI studies, 22% show evidence of active lesions, 78% do not. This contrasts with concurrent studies using interferon where 56% show active lesions. 2) MRI studies with grades of 3 and 4 glioma reveal that tumor sized has decreased a median of 63% after 6-12 months of treatment. Median survival time of our patients has now exceeded expected survival times. Except for 5 patients who died shortly after going on the study, 17 patients are feeling well and working. 3) Twenty-one patients with Tropical Spastic paraparesis in Jamacia, have shown a slight but minimal improvement. 4) In escalating dose studies in normal volunteers, there are no decrements in mental or physical performance, except at doses above those that would be used in the clinic.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00182-12 ODIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical & Genetic Mechanims of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.C. Williams, Ph.D., Sr. Scientist, NIAID/ODIR, Chief, Intracellular Pathogens Branch, Bacteriology Division, USAMRIID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director, Division of Intramural Research, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD and Bldg 550, Ft. Detrick, Frederick, MD 21702-5011

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PROJECT TERMINATED

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00183-12

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Properties of Coxiella burnetii (Q fever) Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.C. Williams, Ph.D., Sr. Scientist, NIAID/ODIR Chief, Intracellular Pathogens Branch, Bacteriology Division

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director, Division of Intramural Research, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD and Bldg 550, Ft. Detreick, Frederick, MD 21702-5011

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PROJECT TERMINATED

PHS 6040 (Rev. 5/92)

Report of the Clinical Director

National Institute of Allergy and Infectious Diseases

Annual Report, 1992

Over the past year the Office of the Clinical Director, NIAID, has developed a number of organizational, operational, and managerial programmatic changes that we hope will enhance and broaden clinical research conducted intramurally at NIAID.

Operations--Clinical Services

In FY 92 there was a decline in patient census and patient visits on the inpatient units and outpatient units, respectively, from the high activity of FY 90 and 91. The drop in clinical activity can be attributed to program reorganization within the clinical laboratories, and to the abrupt cessation of a single large anti-retroviral drug trial due to the development of viral resistance. Patient census and visit counts are expected to increase and stabilize at roughly the level of the end of FY 91 with new clinical protocols under development.

Patient care on 11 East and 11 West was enhanced by the diversity of backgrounds of the monthly ward attending physicians. Since January, 1991, the NIAID senior staff positions have each attended on the inpatient service, conducting triweekly patient care and didactic rounds. The rotation of NIAID senior physicians to this inpatient service has been viewed very positively by all patient care staff.

The position of the Physician Assistant, an NIAID-led experiment within NIH, has been secured with the establishment of a Physician Assistant job classification by the Office of Personnel Management. The success of the trial of using Physician Assistants on the inpatient units is due, in no small part, to the professionalism of the 3 Physician Assistants currently working on NIAID units, Patricia Steele, Cheryl Talar-Williams, and Jennifer Hahn.

Computer use in patient care and clinical research continues to be a major focus of the program. Desktop access to the CC Medical Information System, MIS, was made available to many users with MacIntosh computers via a connection with the NIAID LAN. The Clinical Center is replacing older VMT MIS terminals with MacIntoshes on the inpatient units and has provided MacIntosh computers for the 11th floor clinic control desk and for the Fellows' Office on 11 West. A prototype clinical research database, called the Clinical Information Management Project (CIMP), is midway in development through a contract with Systex Corporation.

Establishment of a new outpatient facility--NIAID Day Hospital

An additional ambulatory care facility for NIAID was opened in October, 1991, on the 11 West inpatient unit. The 4-chair NIAID Day Hospital was designed to provide an intermediate patient care site for lengthy studies, procedure recovery, and administration of intravenous medications, and in doing so, to have flexible hours responsive to the needs of patients and investigators in a cost-effective manner. A survey conducted after one quarter of operation demonstrated that the NIAID Day Hospital has been rapidly accepted by both patients and patient care staff, and visit counts have increased each month.

NIAID/Clinical Center Management Team

A working group composed of representatives from NIAID and Clinical Center was developed in FY 92 to promote communication and cooperation between the Institute and Clinical Center, and to serve as planning body, with decision-making and resource allocation authority for future initiatives. The NIAID/Clinical Center Management Team meets monthly to review and plan activity related to the clinical program. For example, the Management Team has instituted a review of new clinical protocols designed to quantitate resource use, and evaluates space assignments and staffing levels that are of mutual interest to the Institute and Clinical Center. The NIAID/Clinical Center Management Team should have an increasingly visible role in the management and coordination of NIAID's clinical research effort.

Infectious Disease and Allergy/Immunology Training Programs

The clinical training programs of NIAID in Infectious Diseases and Allergy/Immunology continue to attract well-prepared applicants. Fellows beginning in July 1992 were the first group to have the opportunity to obtain clinical training leading to board-eligibility within our clinical service, but with the possibility of research positions in NIAID's extramural divisions after the first year. This new organization of fellow training was designed to allow broader training opportunities for candidates, as well as equitable access to clinical trainees for all divisions.

The Infectious Diseases Training Program combined its consultation service with the National Cancer Institute, and has completed its first year with an extremely active and interesting service. The Infectious Diseases Training Program has benefited from the establishment of an Infectious Diseases Ad Hoc Committee to provide oversight and establish consensus on many aspects of the program.

Collaboration with Howard University

In late FY 91 and 92, a formal collaboration was developed between NIAID and Howard University, Washington, DC, with the goal of mutually informative exchange of patient care expertise between the two groups. Senior NIAID physicians have attended and conducted patient care rounds on general medicine, infectious diseases, and immunology patients. An attending rotation at Howard University will continue to be offered to NIAID staff members in FY 93. Howard University has sent one fellow for a month-long rotation on NIAID's inpatient service and is making plans for sending others in the coming medical academic year.

Clinical Accomplishments during FY 92

Clinical research proceeded along fronts of prophylaxis and immunotherapy within the intramural clinical program. This included completion of a placebo-controlled trial of a herpesvirus vaccine that is currently in analysis. A candidate HIV vaccine, recombinant gp160, that had been studied in Phase I trials here over the past several years, was used to immunize donors in a syngeneic bone marrow transplant study using discordant HIV+/HIV- twin pairs. Active immunotherapy with studies of gp160 and p24 immunogens were continued. In another trial piloting immune reconstitution in HIV infection, a nef-specific cytotoxic T cell clone was administered to its donor with good tolerance and demonstration of modest anti-HIV activity.

A vaccine for Cryptococcus neoformans has been shown to be immunogenic in a Phase I trial. Studies will be expanded to an HIV positive population in the coming year. Alpha interferon continues to be studied as an immune-based therapy for HIV infection, and has been piloted in the treatment of mastocytosis, as well. Gamma interferon, after being FDA-approved in 1991 for use in chronic granulomatous disease, entered phase IV testing to substantiate its long term effects on phagocytic cells and the inflammatory process. In addition, gamma interferon is being studied for its potential to enhance host defense in patients with a newly identified X-linked susceptibility to Mycobacterium avium intracellulare and for patients with Hyper-IgE-recurrent infection syndrome.

Therapeutic studies were aimed at a variety of disorders. Acyclovir was proven to suppress frequently recurring oral herpes. FIAU demonstrated efficacy in suppressing chronic hepatitis B infection in HIV-infected patients and further studies are underway in immunocompetent hosts. Three new anti-retroviral drugs were studied: a non-nucleoside analogue reverse transcriptase inhibitor, L697,661; an immunotoxin, CD4-PE; and N-acetylcysteine (NAC), a drug which may modify *in vivo* replication of HIV-1. BW566C80 was shown to be an effective therapy for Pneumocystis carinii pneumonia and cerebral toxoplasmosis. A trial of BW566C80 as treatment for cryptosporidiosis and microsporidiosis has been completed and is in analysis. Foscarnet was shown to confer a survival benefit in a group of AIDS patients who received it as therapy for cytomegalovirus retinitis. New studies are underway to look at treatment of Wegener's granulomatosis and Takayasu's arteritis with low-dose methotrexate and glucocorticoids, which were prompted by observations of high relapse rates and long-term toxicities after treatment with glucocorticoids alone. Patients with filarial diseases and leishmaniasis (including veterans of Desert Storm), were referred for evaluation, treatment, and to provide research material for studies on the pathogenesis of parasitic diseases.

Observational studies conducted within the program continue to add to the body of knowledge of a variety of disorders. Chronic fatigue syndrome is being studied from neuroendocrinologic, immunologic, and neuropsychiatric angles. A new clinical syndrome manifested by fatigue and lymphadenopathy and characterized by expansion of a T cell population lacking CD4 and CD8 markers, was described. A recently described clinical entity involving CD4 lymphopenia and opportunistic infections in HIV seronegative individuals has just begun to be studied.

Collaboration with Howard University

In late FY 91 and 92, a formal collaboration was developed between NIAID and Howard University, Washington, DC, with the goal of mutually informative exchange of patient care expertise between the two groups. Senior NIAID physicians have attended and conducted patient care rounds on general medicine, infectious diseases, and immunology patients. An attending rotation at Howard University will continue to be offered to NIAID staff members in FY 93. Howard University has sent one fellow for a month-long rotation on NIAID's inpatient service and is making plans for sending others in the coming medical academic year.

Clinical Accomplishments during FY 92

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Animal Care Branch

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ANIMAL CARE BRANCH

1992 Annual Report

Summary

Much of the current research in the understanding and treatment of allergies and infectious diseases requires the use of laboratory animals. The care and treatment of these animals can have a profound affect on experimental results. An animal's environment, its daily care, the presence or absence of disease-causing microorganisms, the amount of pain or distress that an animal experiences may affect the validity of research data generated through the use of animals. The NIAID intramural scientists today are very sensitive to these factors and their ethical responsibility for ensuring that animals used in research receive high quality care. All NIAID scientists conducting research that involves animals are required to attend the NIH course on "Using Animals in Intramural Research: Guidelines for Investigators." This course offers not only a broad perspective on current animal issues, but also provides specific information on actual techniques and procedures.

It is the responsibility of the NIAID Animal Care Branch to provide daily care to all animals maintained within the NIAID animal facilities in Bethesda, Maryland, to oversee NIAID's intramural contracts involving animals, and to assist NIAID scientists with animal-related problems. The NIAID maintains five animal facilities at the NIH campus in Bethesda, Maryland; one animal facility in Rockville, Maryland; two animal facilities at the Frederick Cancer Research Development Center in Frederick, Maryland; and four animal facilities at Rocky Mountain Laboratories in Hamilton, Montana. Approximately 58,000 square feet of space (26,000 sq. ft. at the NIH in Bethesda, MD; 2,500 sq. ft. at the FCRDC in Frederick, MD; and 29,000 sq. ft. in Hamilton, MT), excluding pasture land, are devoted to the NIAID Animal Care Program.

The NIAID's Animal Care Branch provides guidance to the Institute's intramural scientists using animals in research projects. This guidance includes assistance in the purchase of animals, the selection and proper administration of anesthetics and analgesics, the diagnosis, treatment and control of infectious agents, and the performance of technical procedures in laboratory animals. The Branch maintains production colonies of over sixty different strains of mice, hamsters, cotton rats, and rabbits for NIAID investigators within DIR or contract animal facilities. Many of these animals are unavailable anywhere else in the world or are available only after long delays. The NIAID Division of Intramural Research is committed to the use of animals only when alternative methods are unavailable, and fully supports existing Federal rules and regulations pertaining to the care and use of animals in biomedical research. The Institute has four full-time veterinarians to help manage its' animal care and use program and has spent, in FY91, over $1,000,000 to upgrade its' animal facilities.

ADMINISTRATIVE MANAGEMENT BRANCH

ANNUAL REPORT FY 1992

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ADMINISTRATIVE MANAGEMENT BRANCH, DIR
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1991, to September 30, 1992

INTRODUCTION

The Administrative Management Branch (AMB) is charged with the responsibility of planning, directing and coordinating administrative management functions for the Division of Intramural Research (DIR), NIAID. These functions include personnel; space, facilities and financial management, procurement-and travel regulations.

PERSONNEL

During FY92, the AMB experienced an influx of new staff, replacing two Senior Administrative Officers, one Administrative Assistant, and a number of purchasing agents. Additionally, the AMB was reorganized to include the establishment of a Business Management Section at the Rocky Mountain Laboratories, which transferred the administrative and business functions from the Rocky Mountain Operations Branch into the AMB.

The AMB was the initiator of a new Career Development and Exchange Program, a three-part program, which will allow the business Sections and Branches within the NIAID to (1) recruit and hire staff; (2) professionally develop trainees and NIAID staff; and (3) allow exchange of administrative staff members, thus providing opportunities for employees to cross-train and gain a broader view of the Institute's activities and experience in related disciplines, such as budget, personnel, general administration, management, program analysis or grants and contract management.

BUDGET AND FINANCIAL MANAGEMENT

In FY92 the DIR budget allocation was increased by 4.2 percent over FY91, to a total budget of $119,002,000 Forty percent was allocated in support of AIDS and AIDS related research. The AMB is responsible for monitoring and accounting of these funds in each specific research category.

With the increased NIH-wide attention focused on outside activities and related sources of funding, new administrative procedures and controls were implemented to strengthen oversight in this area. AMB established a new data base to provide tracking of these outside activities, sponsored travel, FAES-managed grant funding, CRADA funding, as well as Royalty income. In addition to alerting AMB to potential conflict of interest situations, the system allows for monitoring of the level of expenditures in these areas, and determination of the overall activity level.

RENOVATIONS AND SPACE RELOCATIONS

The AMB was involved in several major renovations and relocations during FY92.

AIDS Facility: Building 10A Wing

Renovation of the Building 10A wing AIDS facility was accomplished during FY92. Staff from the Laboratory of Immunoregulation was reassigned to the 6th floor, and members of the AMB and Office of the Scientific Director were relocated to the 4th floor. This shifting of space will allow expansion of lab areas into vacated space in the 11C and 11N corridors of Building 10.

Twinbrook II

The Phase 1A renovation of Twinbrook II to provide Library, Seminar, and Office space is continuing. Anticipated award of the construction contract is slated for October 1992, with beneficial occupancy in early 1993.

The Phase 2 first floor laboratory renovations are at the 30 percent design stage. This phase of renovation includes a new Structural Biology Laboratory, a Gene Knockout Laboratory and central support space for the facility. Expansion in the Animal Care Branch at Twinbrook II will include a walk-in, high vacuum sterilizer to meet the new requirements related to the care of "clean" rodents. Completion of this renovation is expected in the summer of 1993.

The Twinbrook II Facility administrative staff, along with members of the LAN team, have begun design of a DIR-wide Space Reporting System, which will allow greater flexibility and access to space management data within the laboratories and administrative offices. This system will also assist in the immediate identification of data problems relative to the NIH Space Management System, and enable AMB to prepare for reports of the annual Management Fund Assessment.

Twinbrook I

DIR is in process of creating space at Twinbrook II to accommodate a new Gene Knockout Laboratory. Because of this renovation, it was necessary to consolidate contract staff supplying primers and other research support services to the DIR, to the Twinbrook I facility.

Building 4

In order to allow expansion of the Biological Resources Branch in Building 4, the AMB was able to provide approximately 100 square feet of space for a tissue culture laboratory. In addition, AMB was able to furnish the Laboratory of Viral Diseases with 318 square feet of space to provide computer support for members of their staff.

Building 7

In the Fall 1992, the Hepatitis Viruses Section, Laboratory of Infectious Diseases, began construction of a new, biologically clean P2/P3 laboratory area to study the identification and cloning of both Hepatitis Virus C and Hepatitis Virus E by using Polymerase Chain Reactions (PCR) techniques.

In addition, Building 7 has been chosen to be one of the coordination points for the new, NIH-wide Local Area Network. This new network will enhance communications throughout the entire campus.

PROCUREMENT/CONTRACTS

The Administrative Office located at Twinbrook II provides funding documentation for contract award, renewals, option year election and modifications for procurement contract support. In addition, this office assists scientists with the preparation of statements of work and supporting materials, schedules technical and business evaluation for the renewal process, and is generally responsible for providing overall support in the procurement contract process. One contract award occurred in 1992, and nine additional awards are pending award by the end of CY92. Project SIDA, located in Zaire, Africa, came to a close in FY92. The AMB played a significant role in closing out the project which included transport of equipment, specimens and staff.

In order to better serve the laboratories and AMB purchasing agents, the AMB installed a modern Microfiche system to allow ease of source selection and availability of products. The benefit of this equipment is reflected in the significantly reduced number of orders returned from Central Procurement for additional information and expedited handling of large equipment orders, well within procurement deadlines. In an effort to improve procurement for the NIAID scientists, the AMB instituted a new policy whereas staff who wished to place an order, but had no preference of source, are able to simply enter an order in AMBIS stating "Any Source." This resulted in savings of time and effort for all laboratory staff.

Overall, procurement audits conducted by both PHS and NIH in FY92 were complimentary in nature, and in fact, our organization was commended for its purchasing practices. In addition, the AMB played a significant role in the issuance of two Information and Instruction Memoranda: one relating to proper procedures for Internal Procurement Reviews and the other dealing with certification of procurement officials.

A Procurement Analyst, well-versed in all phases of procurement and contracts, was detailed to the AMB from the Clinical Center in the summer 1992 to assist in the identification and establishment of future indefinite delivery contracts in DIR. Procurement staffs throughout NIH are now required to consolidate government buys whenever possible, and this detail facilitated in the verification of several areas where consolidated purchasing would be possible.

AUTOMATION

During FY92, automation in the AMB/DIR continued to expand, either through the contract mechanism or the NIAID Information Technology and Information Branch. The DIR currently has nearly 800 Local Area Network (LAN) users. Clients have a variety of programs available to them on the LAN, including Status of Funds, AMBIS, the Employee Data Base system, e-mail, WordPerfect, Quattro, Lotus, News and Information, and various other administrative programs to streamline day-to-day operations.

A new Employee Data Base System (EDS) was developed and implemented in FY92 which enables the AMB access to various personnel information on all DIR employees. This system allows the generation of FTE, ceiling and usage reports for the laboratories, and lists an array of data on each employee such as name, title, grade, series, education, training, date information, etc. This system is easy to use and has created an efficient personnel tracking system for the DIR.

A clinical information management project, instituted in 1991, is under development to provide enhanced monitoring of clinical trials and protocols. The project is being implemented in a staged approach to meet the variety of requirements for AIDS patient protocol tracking and overall administration. This is a necessary tool for determining the cost of each active clinical protocol from inception to completion. This program provides the Institute with a clearer picture of its clinical expenditures.

The LAN in the new 10A AIDS Facility was installed in FY92 as part of the NIH telephone cable plant. This translates into newer LAN technologies and reduces the overall cabling costs by combining voice, modem, and LAN labor costs into one function. Use of new twisted-pair (modular) cable will ensure a great degree of troubleshooting and greater longevity of the LAN cable system.

In conjunction with the establishment of the Business Management Section, AMB, RML, resource management programs such Status of Funds and Employee DataBase System were fully implemented at RML. This has significantly increased the control of resources at RML by providing up to date information to the administrative staff. In addition, RML is now participating in a pilot program for the IMPACT System, an NIH system which automates personnel documentation and data.

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Annual Report
Biological Resources Branch
National Institute of Allergy and Infectious Diseases
October, 1, 1991 to September 30, 1992

RESEARCH PROGRESS

The Biological Resources Branch (BRB) investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and hemopoietic differentiation. Special emphasis is placed on membrane receptors and differentiation antigens present on T and B lymphocytes, on the structures of major histocompatibility genes and proteins, on the nature of the peptides bound by class I and class II molecules, on the molecular basis by which antigens are processed and presented to T cells, on the *cis* elements and *trans* factors that regulate T cell receptor and class I gene expression and on proteins of the complement system. In addition to this fundamental immunological research, the BRB provides protein sequencing, peptide synthesis, and flow cytometry expertise and services for the DIR.

STUDIES ON THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules and T cell receptors are emphasized in this research.

CD4$^+$ and CD8$^+$ CTL Recognize Overlapping Epitopes. Viral epitopes that are recognized by both HLA class I-restricted (CD8$^+$) and class II-restricted (CD4$^+$) T cells have been defined for the type A influenza virus nuceloprotein (NP) peptide. CD8$^+$ and CD4$^+$ CTL lines were generated against a synthetic peptide encompassing residues 335-349 of NP that are restricted by HLA-B37 and HLA-DQw5 respectively. Amino acid residues that are critical for recognition of this NP determinant in the context of HLA-B37 and HLA-DQw5 were investigated by the use of panels of truncated and alanine-substituted NP peptides. It was shown that these class I- and class II-restricted CTL lines recognize similar but distinct epitopes, and that different structural features of the NP peptides are required for presentation by HLA-B37 and HLA-DQw5. Comparison of the amino acid sequences of the NP peptide presented by HLA-B37 and HLA-DQw5 with other peptides known to be presented by both class I and class II molecules revealed a common motif among these peptides.

Different Peptides Bind Common Class I Structural Features. To determine whether similar or dissimilar molecular features of class I molecules are involved in the presentation of structurally distinct peptides, we have investigated the influence of different pockets of the HLA-A2.1 molecule on the presentation of three different viral peptides. HTLV-I Tax peptide 12-19, HCMV gB 619-628, and influenza M1 58-66 are minimal peptides that induce HLA-A2.1 restricted non-crossreactive CTL. A detailed analysis of the structural features of HLA-A2.1 that are involved in peptide presentation was undertaken using a panel of 11 HLA-A2 mutants with single amino acid substitutions within pockets present in the peptide binding site. Nine of the 11 mutants affected presentation of each of the three peptides, where the other two mutants had negative effects on presentation of only two of these viral peptides. These results indicate that common structural features in HLA-A2 determine the binding of different peptides, and help provide a plausible explanation for how structurally diverse peptides bind to HLA-A2.

Reconstitution of Class I Molecules from *E. coli* Expressed Proteins. The specificity of peptide binding by human leukocyte antigen (HLA) class I molecules was investigated in a cell-free direct-binding assay. Peptides were assessed for binding to HLA-A2 and HLA-B27 by measuring the formation of heterotrimeric HLA complexes that consisted of iodinated β_2-microglobulin, HLA heavy chain fragments isolated from the *E. coli* cytoplasm, and peptide. No detectable HLA heavy chain-β_2-microglobulin complexes were formed unless appropriate peptides were added to the reconstitution solution. Analysis with monoclonal antibodies demonstrated that these heterotrimeric complexes were correctly folded. Five nonhomologous peptides, known to form complexes with HLA-A2 or HLA-B27 from T-cell functional studies, were tested for their capacity to bind HLA-A2 and HLA-B27 using the reconstitution assay. Four of the peptides bound to the appropriate class I molecule only and one bound to both HLA-A2 and HLA-B27. The optimal length for peptide binding was 9 to 10 amino acid residues; however, one peptide that bound to HLA-B27 was 15 amino acids long. All peptides that bound to HLA-B27 in the direct-binding assay also competed with antigenic peptides for binding to HLA-B27 on the surface of intact cells as determined by a standard cytotoxic T-lymphocyte functional assay. Thus, we concluded that HLA-A2 and HLA-B27 bind distinct but partially overlapping sets of peptides and that, at least in vitro, the assembly of HLA heavy chain-β_2-microglobulin complexes requires specific peptides.

Characterization of Myelin Basic Protein (MBP) Specific T Cell Receptors. Multiple sclerosis (MS), a human demyelinating disease, is thought to be caused by an autoimmunogenic process, and myelin basic protein (MBP) is considered a likely autoantigen with amino acid residues 87 through 106 containing an immunodominant epitope of MBP. In this study, the fine specificity of 29 $CD4^+$ cytotoxic CTL lines that had been generated against whole MBP and were derived from four MS patients and two healthy relatives was dissected using truncated and alanine-substituted derivatives of the 87-106 peptide. In addition, the TcR α and β chain usage of 15 $CD4^+$ CTL was determined. It was shown that the $CD4^+$ cytotoxic T cell response to the immunodominant MBP peptide 87-106 demonstrates a high degree of heterogeneity at the level of fine specificity and TcR usage. These findings indicate that specific immunotherapies aimed at the TcR in MS will probably be more complicated than previously anticipated.

Class II Chain Assembly. This study attempted to identify the post-translational defects responsible for the reduced surface class II molecule expression for both interisotypic α/β combinations that fail to give rise to any detectable cell membrane molecules (e.g., $E\alpha^dA\beta^k$) and intraisotypic pairs with inefficient surface expression (e.g., $A\alpha^dA\beta^k$). The data was consistent with active or passive retention of relatively stable and long-lived mismatched dimers in a pre-*medial*-Golgi compartment, possibly in the endoplasmic reticulum itself. This retention accounts for the absent or poor surface expression of these α/β combinations, and suggests that conformational effects of mismatching in the NH_2-terminal domain result in a failure of class II molecules to undergo efficient intracellular transport.

MOLECULAR STUDIES ON γδ T CELL RECEPTORS AND ACCESSORY MOLECULES

Characterization of Vγ1.1Cγ4/Vδ6(Vδ4) Receptors that Recognize Autoantigens. We have compared the functional properties of Cγ4+ hybridomas derived from newborn thymocytes and adult spleens with $C\gamma4^+$ dendritic epidermal T cells (DETC) in terms of the structural features of their TcRs, their ability to secrete cytokines spontaneously and their the use of the vitronectin receptor (VNR) as an accessory molecule. The thymic hybridomas secreted lymphokines spontaneously and were dependent on the VNR for this activity, whereas the splenic hybridomas failed to secrete lymphokines. Five thymic hybrids expressed the $V\gamma1.1C\gamma4/V\delta6$ receptor and one thymic hybrid expressed a $V\gamma1.1C\gamma4/V\delta4$ receptor that had a close structural relationship to the DETC γ/δ TcR associated with spontaneous lymphokine secretion. The strong correlation of functional and molecular properties between thymocyte $C\gamma4^+$ hybridomas and $C\gamma4^+$ DETC suggests that the $C\gamma4^+$ DETC may be of thymic origin and that cells with potential for autoreactivity residing in the thymus at birth may populate other peripheral locations in the mouse. To further evaluate the role of the TcR, the VNR and the putative autoantigen in the activation of the $C\gamma4^+$ T cell subset, we cloned complete cDNA encoding the $V\gamma1.1C\gamma4$ and $V\delta6C\delta$ TcR and transfected these cDNA constructs into a TcR^- murine hybridoma and into a TcR^- variant of the human Jurkat line. The murine transfectant spontaneously produced IL-2 in culture and IL-2 production could be inhibited by anti-CD3, anticlonotypic mAb to the transfected TcR, and anti-VNR mAb, as well as by RGDS. These results demonstrated that transfection of the $\gamma\delta$ TcR confers to recipient T cells the phenotype of constitutive activation, as well as dependence on engagement of the VNR as an accessory molecule. In contrast, the Jurkat $\gamma\delta$ transfectant failed to produce cytokines spontaneously. Surprisingly, neither the murine transfectant nor the human transfectant could be induced to respond to autoantigen-bearing cells in coculture assays. One interpretation of these results is that coexpression on the surface of the same cell of the $V\gamma1.1C\gamma4V\delta6$ TcR, the VNR, and a putative autoantigen is necessary for T cell activation in this system.

$\alpha_6\beta_4$ Integrin is a Thymocyte Developmental Antigen. Integrins are a superfamily of $\alpha\beta$ heterodimers, most of which serve as cell surface receptors for extracellular matrix proteins. We demonstrated that the recently described $\alpha_6\beta_4$ integrin, previously thought to be limited to epithelial cells and Schwann cells, is expressed on immature mouse thymocytes. The presence of $\alpha_6\beta_4$ is controlled by regulation of β_4 expression. During fetal ontogeny, β_4 was highly expressed by day-13 to day 15 thymocytes, then rapidly declined to low levels by birth. In neonates and adults, β_4 expression was highest on $CD4^-$ $CD8^-$ $CD3^-$ and TcR^+-$\gamma\delta$ subsets. Overall, down-regulation of β_4 was associated with up-regulation of CD4, CD8, and CD3 in the thymus. The data suggest that $\alpha_6\beta_4$ is up-regulated after pro-T cells enter the thymus and may have a thymus-specific function for T cells.

PROTEINS OF THE COMPLEMENT SYSTEM

Biochemical Characterization of the Plasma Sialoglycoprotein. Studies are in progress to characterize the biochemical and biological properties of a plasma sialoglycoprotein (sgp120) that we first identified by its capacity to interact with the activated fourth component (C4b) of the classical complement pathway (CCP). Results to date support the idea that one form of this protein regulates the activation of the CCP. Sgp120 is also cleaved by kallikrein of the intrinsic coagulation-kinin generating pathway (ICKP) to produce protein fragments that may possess biological activity. To obtain unequivocal data to support sgp120 as a new plasma protein and to gain information on its primary structure and function, we are completing studies to clone the gene responsible for its synthesis.

SYNTHESIS OF PEPTIDE ANTIGENS

During the past year, over 540 peptides have been produced by the Branch largely for preparing peptide antisera and for defining MHC class I or class II restricted T cell epitopes. Proteins for which peptides have been provided to make antisera include plasma sialoglycoprotein (Sg p120); IL-8; HB9, a human homeodomain containing protein; *P. faciparum* G6PD, tubulin and Duffy receptors; CD4; SIV; Herpes simplex virus I; IL-10; C-jun; CD3 proteins; CD40; Igmα; *P. gallinaceum* pgs 28; and a human neutrophil G-protein.

Sources of proteins for which peptides have been provided to define T cell epitopes include HIV, HTLV-1, CMV, murine leukemia viruses, influenza viruses, myelin basic protein, Vaccinia proteins, Sendai virus, MCMV, respiratory syncytial virus, and VSV. For functional studies, peptides have been provided that correspond to regions of HIV REX protein, and the HIV TAT protein, that can act as substrates for protein kinases, and that can mimic neutrophil NADPH oxidase activity.

Within the Branch, synthetic peptides have to be used to prepare antisera to a variety of integrins and to define the binding specificity of HLA-A2, HLA-B27, HLA-A3 and HLA-DQw5 molecules. Moreover, peptide epitopes recognized by patient's sera were defined for HTLV-I and HTLV-II. In some cases, these reactives can be used to distinguish HTLV-I from HTLV-II infection.

PROTEIN ANALYSES

The BRB determined the amino acid sequence of about 600 samples. About 250 of these analyses were for unknown proteins or peptides, and the remainder were for quality control of synthetic peptides. Most of the unknown sequences were determined for endogenous peptides eluted from mouse d and b haplotype molecules (LI, D. Margulies) and from human HLA-A3, -A2 and -B27 (BRB). From these analyses, amino acid residues in peptides that are requisite for peptide binding to particular class I molecules have been determined. This information allows one to search protein sequence databanks for potential antigenic T cell epitopes that are recognized by cytotoxic T lymphocytes, which has important implications for vaccine development. In addition, proteins from a large variety of other sources have been sequenced in the facility. These include a 28Kd coat protein from *P. gallinae* and the 25 Kd ookinetic surface antigen from *P. falciparum* (Kaslow, LMR); vaccinia proteins (Moss, LVD); dengue viral proteins (Lai, LID); various glycosidases from *C. albicans* (Williamson, LCI); Il-2 precursor fragment (Kaliner, LCI); eosinophil proteins (Rosenberg, LHD); Duffy blood group antigen (Miller, LMR); TGFβ (Sporn, NCI); and tumor associated antigens (Seon, Roswell Park Memorial Inst.).

Endothelins in primary rat astrocytes were shown to be subject to selective autoregulation, as demonstrated by the potentiation of endothelin-1 secretion after activation of glial endothelin receptors.

FLOW CYTOMETRY ANALYSES

The FCS has recently acquired a surplused FACStar, 4 parameter flow cytometer. This instrument is capable of both analysis and sorting which should help with the increased demand for sorting time seen in FY '92 (50% compared with 38% in FY '91).

New computer hardware and software for our Micro VAX 3400 has been purchased and will be installed this FY in order to provide new Microsoft Windows compatible list mode analysis software as a networked program. In addition, new disk drives will be installed to permit longer term access to data and to meet the increased demand for list mode collection.

Administrative Report

This period marks the fifth year of the existence of the Biological Resources Branch. During this period the Protein Sequence and Synthesis Section was expanded to include Dr. Jan Lukszo, a Visiting Scientist and Peptide Chemist, who previously worked for PRI on the peptide synthesis contract. In addition, Jennifer Martin, a Peptide Chemist, was recruited to assist Dr. Lukszo in making synthetic peptides. Mark Garfield temporarily left of August. Changes to the Facility instrumentation/computers included acquisition of a FACStar flow of Pharmacology, University of Wisconsin ed by Michael Knierman that should greatly enhance the peptide analysis capabilities of the Section.

The Flow Cytometry Section has undergone the following changes during the past year. Mr. Jeff Hooley, operator of the FACStar Plus, left in August, 1991, and was replaced by Calvin Eigsti in December, 1991. We have recently hired Ms. Ruth Swofford to replace Ms. Fran Hausman, operator of the FACS 440, who will leave the FCS at the end of August. Changes to the Facility instrumentation/computers included acquisition of a FACStar flow cytometer, addition of a 7th parameter to the FACStar Plus, the purchase of a larger, 1.2 byte disk drive for the MicroVAX 3400, and the purchase of software upgrades for the MicroVAX which allows Windows-based PC emulation.

The Molecular Immunology Section constitutes the research arm of the Branch. During the past year, Dr. Antonello Punturieri departed to assume a research position at the Regina Elena Cancer Institute in Rome, Italy. Dr. Yumiko Shirakata was promoted from Special Volunteer to Visiting Associate. Recent additions to the Laboratory include Dr. Michael Shields from the Department of Cell, Molecular, and Structural Biology, Northwestern University and Dr. Stephanie Otto from the Department of Pharmacology, University of Wisconsin in Madison. Dr. William Magner from the Department of Biochemistry, Case Western Reserve University will join the Branch in September. Patricia Hong was hired as a technician to replace Jeff Marine.

Honors and Awards

Dr. Coligan was invited to present research seminars at the Centers for Disease Control in Atlanta, The University of Florida, Roswell Park Memorial Cancer Institute and the Uniformed Services University of Health Sciences. He was also invited with expenses paid to speak at the H2/HLA Workshop held on Jekyll Island Georgia and he made a presentation at the FASEB meeting in Anaheim. Dr. Parker chaired the session on Peptide Binding Specificity of MHC molecules at the FASEB Meeting. Dr. Coligan continues to serve on the editorial boards of Current Protocols in Immunology, Molecular Immunology, and Immunologic Research. Dr. Coligan serves as Co-Chairperson on the Program Subcommittee for the Major Histocompatibility Complex block for the Annual AAI meeting with FASEB and serves on the Immunology and Immunotherapy Study Section of the American Cancer Society. Dr. Coligan received recognition from the NIAID for receipt of the PHS Superior Service Award.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00169-15 BRB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: John E. Coligan, Ph.D., Branch Chief, BRB/NIAID

Others:

K. Parker	Senior Staff Fellow	BRB/NIAID
M. DiBrino	IRTA	BRB/NIAID
Y. Shirakata	Visiting Associate	BRB/NIAID

COOPERATING UNITS *(if any)*
Molecular Immunology Section, NINDS (W. Biddison)

LAB/BRANCH
Biological Resources Branch

SECTION
Molecular Immunology Section

INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	2.8	1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The specificity of peptide binding by human leukocyte antigen (HLA) class I molecules was investigated in a cell-free direct-binding assay. Peptides were assessed for binding to HLA-A2 and HLA-B27 by measuring the formation of heterotrimeric HLA complexes that consisted of iodinated β_2-microglobulin, HLA heavy chain fragments isolated from the *E. coli* cytoplasm, and peptide. No detectable HLA heavy chain-β_2-microglobulin complexes were formed unless appropriate peptides were added to the reconstitution solution. Analysis with monoclonal antibodies demonstrated that these heterotrimeric complexes were correctly folded. Five nonhomologous peptides, known to form complexes with HLA-A2 or HLA-B27 from T-cell functional studies, were tested for their capacity to bind to HLA-A2 and HLA-B27 using the reconstitution assay. Four of the peptides bound to the appropriate class I molecule only and bound to both HLA-A2 and HLA-B27. The optimal length for peptide binding was 9 to 10 amino acid residues; however, one peptide that bound to HLA-B27 was 15 amino acids long. All peptides that bound to HLA-B27 in the direct-binding assay also competed with antigenic peptides for binding to HLA-B27 on the surface of intact cells, as determined by a standard cytotoxic T-lymphocyte functional assay. Thus, we concluded that HLA-A2 and HLA-B27 bind distinct but partially overlapping sets of peptides and that, at least in vitro, the assembly of HLA heavy chain-β_2-microglobulin complexes requires specific peptides.

Distinct combinations of class II major histocompatibility complex (MHC) α and β chains show widely varying efficiencies of cell surface expression in transfected cells. Previous studies have analyzed the regions of the class II chains that are critically involved in this phenomenon of variable expression and have shown a predominant effect of the NH_2-terminal domains comprising the peptide-binding site. Post translational defects were examined that are responsible for this variation in surface class II molecule expression for both interisotypic α/β combinations failing to give rise to any detectable cell membrane molecules (e.g., $E\alpha^dA\beta^k$) and intraisotypic pairs with inefficient surface expression (e.g., $A\alpha^dA\beta^k$). The data was consistent with active or passive retention of relatively long-lived mismatched dimers in a pre-*medial*-Golgi compartment, possibly in the endoplasmic reticulum itself. This retention accounts for the absent or poor surface expression of these α/β combinations, and suggests that conformational effects of the mismatching in the NH_2-terminal domain results in a failure of class II molecules to undergo efficient intracellular transport.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00172-14 BRB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis to Peptide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Branch Chief, BRB/NIAID

Others:

C. Hammer	Senior Investigator	BRB/NIAID
J. Lukszo	Visiting Scientist	BRB/NIAID

COOPERATING UNITS *(if any)*

Center for Disease Control (Dr. Renu Lal); NICHHD (Dr. K. Ozato); NIHBL (Dr. W. Leonard)

LAB/BRANCH

Biological Resources Branch

SECTION

Protein Analysis and Synthesis Section

INSTITUTE AND LOCATION

NIAID/NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.1	1.8	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year, over 540 peptides have been produced by the Branch largely for preparing peptide antisera and for defining MHC class I or class II restricted T cell epitopes. Proteins for which peptides have been provided to make antisera include plasma sialoglycoprotein (Sg p120); Il-8; HB9, a human homeodomain containing protein; *P. faciparum* G6PD, tubulin and Duffy receptors; CD4; SIV; Herpes simplex virus I; Il-10; C-jun; CD3 proteins; CD40; Igmα; *P. gallinaceum* pgs 28; and a human neutrophil G-protein.

Sources of proteins for which peptides have been provided to define T cell epitopes include HIV, HTLV-1, CMV, murine leukemia viruses, influenza viruses, myelin basic protein, Vaccinia proteins, Sendai virus, MCMV, respiratory syncytial virus, and VSV. For functional studies, peptides have been provided that correspond to regions of HIV REX protein, and the HIV TAT protein, that can act as substrates for protein kinases, and that can mimic neutrophil NADPH oxidase activity.

Within the Branch, synthetic peptides have to be used to prepare antisera to a variety of integrins and to define the binding specificity of HLA-A2, HLA-B27, HLA-A3 and HLA-DQw5 molecules. Moreover, peptide epitopes recognized by patient's sera were defined for HTLV-I and HTLV-II. In some cases, these reactives can be used to distinguish HTLV-I from HTLV-II infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00352-10 BRB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Branch Chief, BRB/NIAID

Others:

K. Sturmhoefel	Visiting Fellow	BRB/NIAID	A. Punturieri	Visiting Associate BRB/NIAID
G. Kikuchi	Sr. Staff Fellow	BRB/NIAID		
A. Chang	IRTA	BRB/NIAID		
S. Wadsworth	Staff Fellow	BRB/NIAID		
M. Halvorson	IRTA	BRB/NIAID		
S. Otto	IRTA	BRB/NIAID		

COOPERATING UNITS *(if any)*

Laboratory of Immunology, NIAID (Dr. Ethan Shevach)

LAB/BRANCH

Biological Resources Branch

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.8	6.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have compared the functional properties of Cγ4+ hybridomas derived from newborn thymocytes and from adult spleens with $C\gamma4^+$ dendritic in epidermal T cells (DETC) in terms of their structural features, ability to secrete cytokines spontaneously and for the use of the vitronectin receptor (VNR) as an accessory molecule. The thymic hybridomas secreted lymphokines spontaneously and were dependent on VNR for this activity whereas the splenic hybridomas failed to secrete lymphokines. Five thymic hybrids expressed the Vγ1.1Cγ4/Vδ6 receptor and one hybrid a Vγ1.1Cγ4/Vδ4 receptor that had a close structural relationship to the DETC γ/δ TcR associated with spontaneous lymphokine secretion. The strong correlation of functional and molecular properties between thymocyte $C\gamma4^+$ hybridomas and $C\gamma4^+$ DETC suggests that the $C\gamma4^+$ DETC may be of thymic origin and that cells with potential for autoreactivity residing in the thymus at birth may populate other peripheral locations in the mouse. To further evaluate the role of the TcR, the VNR and the putative autoantigen in the activation of the $C\gamma4^+$ T cell subset, we cloned complete cDNA encoding the Vγ1.1Cγ4 and Vδ6Cδ TcR and transfected the cDNA constructs into a TcR$^-$ murine hybridoma and into a TcR$^-$ variant of the human Jurkat line. The murine transfectant spontaneously produced IL-2 in culture and IL-2 production could be inhibited by anti-CD3, anticlonotypic mAb to the transfected TcR, and anti-VNR mAb, as well as by RGDS. These results demonstrated that transfection of the γδ TcR confers to recipient T cells the phenotype of constitutive activation, as well as dependence on engagement of the VNR as an accessory molecule. In contrast, the Jurkat γδ transfectant failed to produce cytokines spontaneously. Surprisingly, neither the murine transfectant nor the human transfectant could be induced to respond to autoantigen bearing cells in coculture assays. One interpretation of these results is that coexpression on the surface of the same cell of the Vγ1.1Cγ4Vδ6 TcR, the VNR, and a putative autoantigen are necessary for the T cell activation in this system.

Integrins are a superfamily of αβ heterodimers, most of which serve as cell surface receptors for extracellular matrix proteins. We demonstrated that the recently described $\alpha_6\beta_4$ integrin, previously thought to be limited to epithelial cells and Schwann cells, is expressed on immature mouse thymocytes. The presence of $\alpha_6\beta_4$ is controlled by regulation of β_4 expression. During fetal ontogeny, β_4 was highly expressed by day-13 to day 15 thymocytes, then rapidly declined to low levels by birth. In neonates and adults, β_4 expression was highest on CD4$^-$ CD8$^-$ CD3$^-$ and TcR$^+$-γδ subsets. Overall, down-regulation of β_4 was associated with up-regulation of CD4, CD8, and CD3 in the thymus. The data suggest that $\alpha_6\beta_4$ is up-regulated after pro-T cells enter the thymus and may have a thymus-specific function for T cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00522-05 BRB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Sequence Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Branch Chief, BRB/NIAID

COOPERATING UNITS *(if any)*

LIR/NIAID (Dr. H. Ehrenreich), LCI/NIAID (Dr. P. Williamson), LI/NIAID (Drs. Margulies, Germain, Sitkovsky), NCI (M. Sporn), Roswell Park Memorial Inst. (B. Seon), Dana Farber Cancer Inst. (H. Reiser)

LAB/BRANCH

Biological Resources Branch

SECTION

Protein Analysis and Synthesis Section

INSTITUTE AND LOCATION

NIAID/NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	0.1	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The BRB determined the amino acid sequence of about 600 samples. About 250 of these analyses were for unknown proteins or peptides, and the remainder were for quality control of synthetic peptides. Most of the unknown sequences were determined for endogenous peptides eluted from mouse d and b haplotype molecules (LI, D. Margulies) and from human HLA-A3, -A2 and -B27 (BRB). From these analyses, amino acid residues in peptides requisite for peptide binding to particular class I molecules have been determined. This information allows one to search protein sequence databanks for potential antigenic T cell epitopes that are recognized by cytotoxic T lymphocytes which has important implications for vaccine development. In addition, proteins from a large variety of other sources have been sequenced in the facility. These include a 28kd coat protein from *P. gallinae* and the 25 Kd ookinetic surface antigen from *P. falciparum* (Kaslow, LMR); vaccinia proteins (Moss, LVD); dengue viral proteins (Lai, LID); various glycosidases from *C. albicans* (Williamson, LCI); Il-2 precursor fragment (Kaliner, LCI); eosinophil proteins (Rosenberg, LHD); Duffy blood group antigen (Miller, LMR); TGFβ (Sporn, NCI); and tumor associated antigens (Seon, Roswell Park Memorial Inst.).

Endothelins in primary rat astrocytes were shown to be subject to selective autoregulation, as demonstrated by the potentiation of endothelin-1 secretion after activation of glial endothelin receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI-00523-05 BRB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Flow Cytometric Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Kevin L. Holmes, Ph.D., Branch Chief, Flow Cytometry Section

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biological Resources Branch

SECTION
Flow Cytometry Section

INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, maryland 20892

TOTAL STAFF YEARS: 5.25	PROFESSIONAL: .25	OTHER: 5.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The FCS has recently acquired a surplused FACStar, 4 parameter flow cytometer. This instrument is capable of both analysis and sorting which should help with the increased demand for sorting time seen in FY '92 (50% compared with 38% in FY '91).

New computer hardware and software for our Micro VAX 3400 has been purchased and will be installed this FY in order to provide new Microsoft Windows compatible list mode analysis software as a networked program. In addition, new disk drives will be installed to permit longer term access to data and to meet the increased demand for list mode collection.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-543-05 BRB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Recognition of Peptide Antigens by Virus-Specific Cytotoxic T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Branch Chief, BRB/NIAID

Others:

M. DiBrino	IRTA	BRB/NIAID
M. Shields	IRTA	BRB/NIAID

COOPERATING UNITS *(if any)*

Neuroimmunology Branch, NINCDS (W. Biddison, R. Martin, H. McFarland)

LAB/BRANCH

Biological Resources Branch

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID/NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Viral epitopes that are recognized by both HLA class I-restricted ($CD8^+$) and class II-restricted ($CD4^+$) CTL have been defined for a type A influenza virus nucleoprotein (NP) peptide encompassing residues 335 to 349 of NP that are restricted by HLA-B37 and HLA-DQw5, respectively. Amino acid residues that are critical for recognition of this NP determinant in the context of HLA-B37 and HLA-DQw5 were investigated by the use of panels of truncated and alanine-substituted NP peptides. The results showed that the class I- and class II-restricted CTL lines recognize similar but distinct epitopes, and different structural features of the NP peptides are required for presentation by HLA-B37 and HLA-DQw5. Comparison of the amino acid sequences of the NP peptide presented by HLA-B37 and HLA-DQw5 with other peptides known to be presented by both class I and class II molecules revealed a common motif among these peptides.

Multiple sclerosis (MS), a human demyelinating disease, is thought to be caused by an autoimmunogenic process, and myelin basic protein (MBP) is considered a likely autoantigen. Studies of T cell lines (TCL) responding to different parts of the MBP molecule have indicated that amino acids 87 through 106 contain an immunodominant epitope of MBP. The fine specificity of 29 $CD4^+$ cytotoxic, long term, and limiting dilution TCL that had been generated against whole MBP and were derived from four MS patients and two healthy relatives was dissected using truncated and alanine-substituted peptides for the 87-106 peptide, and the TCR α and β chain usage of 15 $CD4^+$ TCL was determined. The results showed that the $CD4^+$ cytotoxic T cell response to the immunodominant MBP peptide 87-106 demonstrates a high degree of heterogeneity in fine specificity and TCR usage. These findings indicate that specific immunotherapies aimed at TCR in MS will probably be more complicated than previously anticipated.

To determine whether similar or dissimilar molecular features of class I molecules are involved in the presentation of structurally distinct peptides, we used site-directed mutagenesis to investigate the influence of different peptide-binding pockets of the HLA-A2.1 molecule on the presentation of three different viral peptides. HTLV-I Tax peptide 12-19, HCMV gB 619-628, and influenza M1 58-66 are minimal peptides that induce HLA-A2.1-restricted noncrossreactive CTL. These results indicated that common structural features in HLA-A2 determine the binding of different peptides, and help to provide a plausible explanation for how structurally diverse peptides bind to HLA-A2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00595-02 BRB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification, Characterization and Function of Proteins of the Complement System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Carl H. Hammer, Senior Investigator, BRB/NIAID

Others:

Thomas L. Leto, Senior Staff Fellow, LHD/NIAID

COOPERATING UNITS *(if any)*

Michael M. Frank, Chairman, Dept. of Pediatrics, Duke Univ. Med. Ctr., Durham, NC 27710

LAB/BRANCH

Biological Resources Branch

SECTION

Protein Analysis and Synthesis Section

INSTITUTE AND LOCATION

NIAID/NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation is ongoing into the biochemical and functional properties of a plasma sialoglycoprotein of 120 kDa (sgp120-A) that was first identified by isolation (3-5% yield) with the second component of human complement (C2) on "C4b"-Sepharose. The capacity of sgp120 to bind to the galactosyl-specific lectin, Jacalin was used to purify sgp120 at up to 40% yield from plasma which contained both binding and primarily non-C4b binding (sgp120-I) forms. Although the two forms are immunochemically indistinguishable by double diffusion analysis, by a number of criteria these two forms are distinct and in particular sgp120-A possess most of the described classical complement pathway inhibitory activity. We have fully sequenced clone A that produces a fusion protein detectable by monospecific antibody to sgp120. We are in the process of completing the sequence of larger identified clones B=2.0 and C=2.1 kB. Clone D=2.2 kB was fully sequenced and resulted in an open frame translation of 729 amino acids. Fast A database search of all protein sequences for clone D translation confirmed our earlier conclusions of the unique structure of sgp120. The results of this latest search of 113000 sequences, however, did find significant homology (41% identity in a 353 amino acid overlap) with human Inter-alpha-trypsin inhibitor. We have essentially sequenced clones B and C and like clones A and D these also contain the 998 bp 3'-terminal of the sgp120 gene. Like clone B, clones C and D also contain the N-terminal 15 amino acid sequence identified in the 25 kDa peptide as well as the 16 amino acid sequence found in the 35 kDa peptide obtained by kallikrein digestion of plasma purified sgp120. To further search for full length clones of sgp120 we have utilized a probe prepared from the 5' end of clone D. This probe in conjunction with a 1 kB probe prepared from clone A identified two clones 7-1, G & 8-1, F. Preliminary results obtained from sequencing isolated sgp120 cDNA from clones F and G extend the known base sequence of clone D from 2189 bp to 2270 (81 bp) and to 2404 (215 bp), respectively. Bestfit analysis of the open reading from of clone G to the N-terminal of sgp120 did not identify the N-terminal sequence. Thus, a full length gene for sgp120 still needs to be identified to obtain the entire coding sequence that should minimally be between 2.6 to 3.0 kB. Analysis of the internal base sequence of sgp120 cDNA from clones B to G has identified an Internal splice region accounting for an insertion of up to 35 amino acids within D clone (residues #364-388), 30 amino acids within B & G clones and no insert found in C & F clones. This information suggests a rationale for the two forms of Sgp120 described earlier.

LABORATORY OF CLINICAL INVESTIGATION
1992 Annual Report
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Summary Report
Laboratory of Clinical Investigation
October 1, 1991 to September 31, 1992

Stephen E. Straus, M.D.
Chief, Laboratory of Clinical Investigation
NIAID

Introduction

All components of the LCI have thrived for the past year with a steady pace of effort and important research accomplishments in infectious diseases, allergy and immunology. Senior staff continue to rise in international prominence and recognition, with countless invited lectures, appointments, and prestigious awards. LCI senior staff direct both the Allergy/Immunology and Infectious Disease Training Programs. They essentially constitute the Allergy Consult Service and direct and heavily staff the Infectious Disease Consult Service. Over 80% of admissions to the 11th floor clinic and about $^1/_4$ of all admissions to the in-patient units were scheduled and supervised by the LCI. Seven LCI staff members serve in rotation as attending physicians on the in-patient units.

Organizational Resources

FY 92 witnessed further change in the organization and staffing of the LCI, completing its initial phases of revision and paving the way for expansion of selected programs within the Laboratory and recruitment of new senior staff.

Following appointment of the new laboratory chief in 1991, the remaining core of the LCI boasted 5 sections with 7 tenured staff and 7 others at the level of senior staff fellows. By the end of FY 92 there were 5 sections and the new Bacterial Pathogenesis Unit. Among individuals in the senior staff fellow category, Dr. Stephen James left the Mucosal Immunity Section to be Professor, and Head of the Division of Gastroenterology at the University of Maryland. Dr. Parris Burd of the Mast Cell Physiology Section will be leaving for another post and Dr. Robert Hohman of the Allergic Diseases Section will leave by mid 1993. Dr. Martha White continues to serve as the key pediatric allergy staff member. Dr. Michael Sneller is retained to initiate and conduct a clinical program in immunotherapeutics. Dr. Thomas Russo was appointed Head of the Bacterial Pathogenesis Unit with fully autonomous space and budget. Independent space and staff were also designated for Dr. Jeffrey Cohen who has been formally presented to the Director, DIR as a tenure-track candidate.

In FY 92 space allocations to the LCI were revised. The Mucosal Immunity Section will code 3 research modules to the Laboratory of Immunology in return for 3 equivalent, newly renovated adjoining modules currently occupied by members of the administrative branch and OD. The Clinical Mycology Section will vacate it space at the end of the 11 north corridor and occupy renovated space in the 11C100 corridor that is now newly assigned to the LCI. Additional office space was acquired for the Head, Mast Cell Physiology Section and extensive clinic and office space was committed to LCI personnel in the bridge area and within the 11th floor clinic. These, together, will house new offices for the Head of the Clinical Mycology Section, and offices for Dr. Sneller a medical officer in the Medical Virology Section and 3 research nurses. Expanded clinical research space for the Allergic Diseases Section is also committed in the Clinical Director's plan for renovation and reorganization of the 11th floor clinic.

The LCI now occupies expanded common equipment facilities jointly with the Laboratory of Immunoregulation and Laboratory of Host Defenses. There is a new darkroom and film developer and new beta counters and ultracentrifuges.

Research Progress

The **Medical Virology Section** has had an outstanding year and is growing. Already fruitful clinical programs were initiated in several areas. Development of an effective vaccine prophylaxis and immunotherapy of genital herpes is a priority that was approached this past year by the completion of the first trials of a recombinant glycoprotein vaccine for a human herpesvirus infection. Safety was verified and immunogenicity exceeded expectation. Because of this, a placebo-controlled trial was conducted and concluded, with data analysis in process. The past year acyclovir was proven for the first time to suppress frequently recurring oral herpes. A new lymphoproliferative disease was defined this past year in collaboration with Drs. Sneller and Strober. Phase I investigations of a new oral fluoropyrimidine nucleoside analogue, FIAU, showed remarkable potency in suppressing chronic hepatitis B virus infection in HIV-infected subjects. A collaboration was formalized with Drs. Hoofnagle and DiBisceglie of NIDDK to rapidly exploit these observations. Studies were extended to the normal host with chronic hepatitis B virus infection. Marked and possibly permanent suppression of infection can now be achieved with an oral medication at doses lacking obvious toxicity.

Chronic fatigue syndrome continues to be studied in a multidisciplinary manner. A working group was formed to meet every 2 weeks to develop and track research studies that are conducted in collaboration with other universities and with staff of the NICHD, NINDS, NIMH, and PRI Inc. at the FCRC. The LCI reported this past year important original observations regarding neuroendocrinology, immunology, and neuropsychology.

In the laboratory, Dr. Cohen has achieved noteworthy successes in defining the precise domain of the Epstein-Barr virus EBNA-2 protein that the virus must possess to transform B cells. He has shown that a single amino acid change in a critical part of the EBNA-2 protein eliminates EBV's capacity to transform B cells. Dr. Cohen's work on varicella-zoster virus molecular biology has now matured with his first abstracts and submitted publications further defining VZV regulatory gene domains. His major undertaking has been to develop a versatile system for introducing new sequences into VZV or alternatively to knock out specific elements so as to explore these roles in infection. Significant success has been made to this end. He has developed a system to generate infectious VZV DNA cosids.

Dr. Hiroyuki Moriuchi has completed construction of a large collection of cell lines that stablely express individual VZV regulatory genes. VZV genes 4, 10, and 62 were individually inserted into cells and their activity has been explored in detail. Dr. Moriuchi has used these systems to verify the transregulatory activity of VZV genes and he has made the important observation that VZV gene 61 is the functional homologue to the herpes simplex virus ICP0 regulatory gene.

The nature and mechanism of VZV latency in human neural tissues remains a dominant interest of the Section. Detailed in situ hybridization and northern hybridization analyses of trigeminal ganglia from over 100 autopsies have been completed by Dr. Jeffrey Meier. The findings are beginning to fill in a reasonable description of which VZV genes are expressed in latently infected tissues, and in which cell types. To date, we have detected the presence of VZV transcripts in human satellite cells and genes 29 and 62 are clearly expressed. There remains uncertainty about the expression of genes 4 and 63, while genes 28 and 61 are clearly not expressed to detectable levels. Over the coming year, this project will be expanded with the introduction of the formidable new technique of in situ PCR.

A new project in the laboratory has involved the characterization of VZV gene 63 by Dr. Rhonda Kost, in terms of its transcript and activity. Initial findings suggest that gene 63 encodes another viral transregulatory protein. Its promoter has been localized in transient transfection assays.

New staff was recruited to the Medical Virology Section in the last months of the year to expand research efforts, particularly as they regard issues of latency and pathogenesis of varicella-zoster virus and herpes simplex virus. Liyanage Perera, DVM, Ph.D. of the Uniform Services University of the Health Sciences joined the group to extend his prior work on VZV regulatory genes and to initiate a new effort in which animal models will be used to explore the pathophysiolgic impact of genetic alterations in the viral coding sequence. Richard Williams, Ph.D. has also been recruited to initiate a program to study interactions of VZV envelope glycoproteins and the cell surface and hopefully, to identify and clone the human receptor for the virus. He had notable successes in achieving this for the human and murine coronavirus receptors.

Projects being carried out in the **Clinical Mycology Section** are aimed at defining selected aspects of the molecular biology, biochemistry, immunology, treatment, and now also, the prevention of human fungal infections. In this later regard, a major new effort of the Section is the developement and testing of a vaccine for Cryptococcus neoformans. The group is currently exploring 2 vaccine candidates. In the laboratory a 90 kD protein has been isolated from the cryptococcus capsule. The capsular of protein has enzymatic activity; it may be a glucosyl transferase. Importantly, as a constituant of the yeast surface, the protein may be suitable for incorporation into a vaccine. Work on another vaccine candidate has proceeded far further. A conjugate vaccine has been prepared in which cryptococal polysaccharide is coupled to tetanus toxoid. The conjugate vaccine protects mice from lethal infection with Cryptococcus neoformans. An initial phase of clinical trials involving this conjugate vaccine is nearing completion. To date, 50 healthy adults have been vaccinated, twice each, with either of 2 different doses of the conjugate vaccine. Preliminary findings show a 1-2 log increase in titers of anti-cryptococcal polysaccharide antibody. Titers appear to be sustained for at least 3 months. The group is currently designing further clinical trials to assess the immunogenicity of this vaccine in HIV-infected patients. The hope would be to create a vaccine to prevent cryptococcosis, which accounts for the death of about 1 in 12 patients with AIDS.

Dr. Kwon-Chung directs another major series of basic research studies involving Cryptococcus neoformans. Her goal is to define the molecular basis for cryptococcal virulence. It has been known that there are 2 mating types of Cryptococcus neoformans, (alpha) and a. The investigators perform an extensive series of matings and backcrossings to generate strains of cryptococci that are isogeneic but for the gene encoding their mating type. Strains possessing the (alpha symbol) factor proved to be significantly more virulent, in terms of their ability to kill mice, than strains possessing the A mating type. Efforts are now underway molecularly knock out the (alpha symbol) gene to determine whether it is possible to create an avirulent strain of cryptococcus.

Another factor that appears to contribute to the virulence of Cryptococcus neoformans is its expression of the enzyme diphenol oxidase. Mutants deficient in this enzyme lose their virulence. To approach this problem, Dr. Peter Williamson purfied diphenol oxidase to homogeneity. The amino terminus of the protein has been sequenced and oligonucleotides have been synthesized based on the predicted sequence. These oligonucleotides will be used to "fish out" the gene for diphenol oxidase from a cryptococcal recombinant library. The goal would be to clone and express the gene and develop strategies for deleting it from the yeast.

Two research projects are addressing basic questions regarding the biology and pathogenicity of Candida species. Classical genetic and molecular techniques are being used in one series of studies to define the taxonomy and molecular relatedness between various medically important yeasts within the genus Candida. The original goals of the project have been successfully achieved. On an enzymologic basis, 3 pairs of medically important yeast have been considered to be identical despite phenotypic differences among them. Pulse-field gel electrophoresis and hybridization with species-specific probes were performed, showing that there were no distinguishing molecular characteristics of the taxonomically related yeasts. Thus, these modern techniques verified current taxonomic placement of the pathogens.

An exciting project is being spearheaded by Dr. Antonia Geber. She is interested in the nature and role of the Candida albicans maltase gene. Maltase is required by the yeast for metabolism of sucrose and maltose. The gene from maltase has been cloned and sequenced by her and its putative promoter domains have been identified. Functional domains of the enzyme are being targeted by site-directed mutagenesis. By analogy to findings in nonpathogenic yeast, a transregulatory gene CASUC1 has been identified. Future aims of the project are to study the ability of CASUC1 to act on the maltase gene promoter, and to disrupt the maltase gene coding sequence itself so that the effect on yeast survival and virulence could be addressed. Strains of Candida albicans that have spontaneously lost their ability to metabolize sucrose were shown previously to have reduced virulence. The ability to diminish virulence at will, by targeting the gene responsible for sucrose utilization, would provide better insights as to how sugar metabolism permits yeast to be pathogenic and invasive.

Over the past year the **Bacterial Pathogenesis Unit** has expanded its research effort into 3 related project areas. The primary goal remains the determination of the molecular basis for virulence in E. coli. Dr. Thomas Russo began his work by identifying an E. coli clinical isolate associated with a severe bacteremic infection. The bacterial strain was shown to resist killing in high concentrations of human serum. Resistance to killing in serum implies surface features of the bacteria that are able to avoid activation and lysis by complement components in the serum. To identify the specific surface moieties responsible for serum resistance random mutagenesis of the E. coli was performed by inserting a transposable DNA element. This transposan mutagenesis was successful in creating a family of bacteria that were now sensitive to killing in serum. Detailed analysis of the mutants revealed a minimum of 3 genetic loci that are responsible for production of the bacterial capsule. All 3 loci appear to be physically linked. One of the loci has now been cloned and sequenced and shown to possess homology to a capsular transport gene of Neisseria. The specificity of this identification reveals that the strategy to uncover critical surface determinants of bacterial virulence is working.

In a related project, the genetic determinants of E. coli capsule type are being addressed. Bacteria possessing group I capsules possess low virulence; group II capsules lead to highly pathogenic organisms. Although the pathogenic E. coli isolate under study in this laboratory expresses the group II capsule, sequences for both group I and group II capsular polysaccharide were present. It is possible now to explore individually the regulation and expression of each of these gene families. In initial studies Dr. Russo showed that expression of the group I and II capsular polysaccharide genes is somewhat interrelated.

A third project is using techniques well established in this laboratory to study the molecular epidemiology of E. coli strains associated with recurrent urinary tract infections in women. Recurrent urinary tract infection is an enormously frequent and vexing problem of women, particularly during their reproductive years. Current wisdom states that repeated urinary tract infections is this setting are due to serial infections with different organisms carried in the GI tract. The technique of pulse field gel electrophoresis permits a detailed physical "footprinting" of large genomic elements. The pattern generated is specific for each strain of organism. In collaboration with Dr. Walter Stamm, a renowned investigator of recurrent urinary tract infections, Dr. Russo is performing pulse gel electrophoresis on panels of bacterial isolates. Pilot studies suggest that repeated infections are due to the persistence within the women of the identical bacterial electropherotype. If this bears out, strategies to treatment may need to address persistent carriage of the bacteria.

Four major and interrelated projects are being conducted by members of the **Mast Cell Physiology Section**. The major long-term goal is to define the cellular and molecular features and requirements for mast cell growth, development, and action in allergic and inflammatory disorders. Mast cells are the critical determinants of many allergic processes but knowledge of this cell has suffered through difficulty in handling the cells and in cultivating them in vitro. The past few years has witnessed enormous advances towards a versatile and accessible in vitro culture system for mast cells. The studies of mast cell biology in this Section, then, have several components. First, the investigators have documented the specific ability of mouse and human bone marrow cells to undergo differentiation along mast cell and basophil lines in the presence of specific growth factors. From an extensive survey of growth factors, only IL-3 has been shown to be required for differentiation of mast cells from CD34+ human bone marrow stem cells. The stem cell factor c-kit ligand was shown to further potentiate the growth and maturation of mast cells and basophils. Full tools are in hand to define some intermediate stages in mast cell differentiation. These include evolving ultrastructural features on electromicroscopy such as the appearance of granules, and the expression on the cell surface of various classes of Fc receptors for IgG and IgE.

Mast cells that evolve a mature phenotype are found in tissues. This implies that the differentiating mast cell must begin to express adhesion receptors on their surface. To date, specific cell surface receptors for laminin, fibronectin, and vitronectin have been shown on mature mast cells. Studies have shown that binding to laminin is specific to a pentapeptide of the protein and that binding initiates a cascade of intracellular responses leading to alteration in expression of cellular and nuclear regulatory factors.

A related series of experiments address the transcriptional sequelae of mast cell activation. As mentioned above, binding of mast cells to adhesion molecules activates certain genes. A serious survey has been initiated to define the exact type and kinetics of cytokines that respond to mast cell activation. This is being carried out in two ways. First, Dr. Parris Burd is studying the regulation and expression on mast cells of 2 gene products: TCA3 and endothelin. TCA3 is a proinflammatory cytokine produced by both mast cells and T cells in response to activation signals. There is some fundamental knowledge regarding TCA3 expression in activated T cells. By analogy, studies of TCA3 expression in mast cells are being conducted. TCA3 RNA expression appears very early after mast cell activation and persists for only a few hours. Drug-block and nuclear run-on experiments suggest that TCA3 expression is regulated transcriptionally. Parallel studies showed that activated mast cells contain and secrete endothelin-1. Expression occurred several hours after mast cell activation and did not seem directly linked to cellular degranulation.

Cytokine release by activated mast cells is also being explored in an in vivo model. Mice that are passively induced to undergo cutaneous anaphylaxis are studied. This is a local, cutaneous, IgG-mediated, and mast cell-dependent reaction. Analysis of mouse skin at various times following induction of local anaphylaxis has revealed a prompt, concommitant release of IL-6. Other cytokines do not clearly accumulate as part of this process.

The Mast Cell Physiology Section is undertaking 2 areas of clinical investigation. A variety of studies are addressing the nature and basis for adverse reactions to foods and food additives. Despite common claims, the investigators found no evidence that monosodium glutamate caused airway reactivity. In collaborative studies with scientists in India, Dr. Metcalfe has identified the major specific protein of shrimp that causes food allergy. Purification of the antigen may permit improved more standardized diagnosis and better reagents for clinical desensitization.

Allergic eosinophilic gastroenteritis is a rare, very debilitating disorder in which a local severe inflammatory response in the stomach prevents normal digestion and absorption of nutrients. Patients can become quite malnourished as a result. An exploration of immune aspects of this disease revealed an imbalance between the various classes of cytokines that are being expressed in the gastric mucosa and by peripheral blood cells from patients. Release of the cytokines IL-4 and IL-5 from lymphocytes is markedly increased in patients with allergic eosinophilic gastroenteritis but there is no increase in gamma interferon. This is consistent with the enormous eosinophilic response in elevation of IgE in these patients.

Mastocytosis is an uncommon but threatening progressive disorder of children and adults. For over a decade Dr. Metcalfe has headed an intensive clinical and laboratory analysis of this disease. Extensive studies have permitted an improved classification and categorization of disease types. Recent findings have emphasized that mastocytosis associated with hematologic complications or with diffuse invasive mastocytosis are associated with progressive liver disease including fibrosis and ultimately portal hypertension.

Two recent findings of special interest are being further pursued as well. First, an exploration is beginning to determine whether patients with mastocytosis have elevations in IL-3. Since IL-3 is required for mast cell growth and differentiation, one model of mastocytosis would be an excessive production of this cytokine. As a first step, cytokine concentrations are being determined in the fluids of blisters induced in the skin of patients with cutaneous mastocytosis. The second deals with an effort to reproduce and extend a recent observation that recombinant alpha interferon can lead to a partial remission of mastocytosis. In a pilot study the first patient is on this treatment. Should he respond, a protocol to more fully exploit this observation will be undertaken.

Dr. Michael Kaliner and his colleagues in the **Allergic Diseases Section** conduct a series of studies covering 2 general areas. The first, personally directed by Dr. Robert Hohman, involves detailed biochemical investigations of early events in the activation of mast cells in vitro. The second research interest is covered by 3 projects, all of which are focused on defining the normal and abnormal biology of the nasal mucosa.

Studies of the biochemical events following activation of mast cells by IgE have shown these cells to possess a series of pathways analogous to those used by T lymphocytes following their receptor-mediated activation. The group is exploiting a now well established rat basophilic leukemia (RBL) cell model. In response to binding of IgE to the surface, these cells degranulate. The potent immunosuppressive drugs cyclosporin A and FK506 were found to prevent IgE-mediated degranulation of the cells. The kinetics, stoichiometry, and biochemical features of this inhibition are identical to those associated with inhibition of T cell receptor-mediated activation of T lymphocytes. FK506 and cyclosporin A appear to work by binding to intracellular proteins known as immunophilins. Each drug has its own distinct target immunophilin. The drugs rapamycin and 506BD bind to the same immunophilin as FK506. Its use in vitro successfully competed with FK506 for binding and inhibition of mast cell degranulation. In a related series of studies Dr. Hohman and his colleagues documented that activation of mast cells by binding of IgE leads to a displacement of one isoenzyme of phospholipase C from the cytoplasm to the membrane. The translocation is regulated by tyrosine phosphorylation.

Most members of this Section are working on one or more aspects of the biology of the nasal mucosa. The group is completing a multi-year investigation of the constituents of mucus and the factors which regulate their release in health and in disease. Specific protein, enzyme, immunoglobulin, prostaglandin, and neuropeptide concentrations have been determined. A vast literature is emanating from this group regarding each of these many components of mucus. Using a combination of biochemical, histologic, and molecular tools it has been possible to localize the site of concentration of many of these constituents and define which ones are released in response to diverse factors such as neural stimulation. Each of these studies are conducted in a collaborative fashion. The team includes ENT surgeons who acquire precious human turbinate tissues. Some of the work is done in a guinea pig model. Many of the studies require collections of nasal mucus by lavage of volunteers.

The following are among the more important observations made over the past year: A number of components of mucus where shown to derive from mucus glands in response to stimulation by the cholinergic nerves. It is known that there are 5 types of cholinergic nerves based upon the specific class of receptor each possesses. Using newly developed drugs which antagonize individual classes of these nerves, it was possible to show that the M3 class of nerves is most relevant to nasal glandular secretion. That finding correlated well with the demonstration that of 3 major cholinergic nerve types, the M3 type was most abundant in the submucosal glands and adjacent deep blood vessels. In situ hybridization studies showed the presence and distribution of message for the M3 receptor in these glands.

Studies of secretory leukoprotease inhibitor (SLPI) are defining a new defense mechanism resident in the respiratory mucosa. SLPI blocks the effect of proteases. As part of a vigorous inflammatory response, PMNs release large quantities of the protease elastase. Elastase has the capacity to severely injure airway surface. This type of damage can occur in asthma and is thought to be important in cystic fibrosis. SLPI may play a role in protecting the respiratory mucosa from elastase.

Another series of studies in the laboratory explores early events associated with hypersensitivity phenomena. Among these is an analysis of a human neutrophil protein that induces mast cells to release histamine. Efforts are underway to biochemically purify and define the neutrophil histamine releasing factor. Thus far it is known to be a relatively small molecule.

In a curious situation in which ongoing research can quickly define new clinical observation, the investigators have been studying unexpected nasal side effects of patients who are being treated with IL-4 as part of experimental cancer therapy studies. It was observed that patients receiving IL-4 experience profound nasal congestion, sufficient to limit treatment. Members of the Allergic Diseases Section documented in the laboratory and in specimens taken from the IL-4-treated cancer patients that the cytokine causes vigorous and immediate histamine release from cells in the nasal mucosa. This provided a trivial strategy to counteract or prevent the nasal complications of IL-4 treatment.

Ongoing studies within the **Mucosal Immunity Section** can be divided into two general areas: studies of immune mechanisms in the mucosa and lymph nodes, and studies of Ig synthesis and regulation. Each of these, in turn, is studied under the aegis of two research projects. One project continues an investigation of a subject of long standing interest in the Section, namely, inflammatory bowel disease. The hypothesis being examined is that inflammatory bowel diseases derive from abnormal immune responses to mucosal antigens. The current studies are addressing this by examining the expression of the TCR-Vβ family repertoire. Over 20 TCR-Vβ families are defined. Abnormal responses to an antigen or superantigen would lead to relative alterations in the expressing of one of the Vβ families or all of them. The strategy to detect this involves a quantitative RT-PCR analysis of individual Vβ mRNAs extracted from lamina propria and comparing the levels to those of the patients' peripheral blood mononuclear cells. To conduct this in a meaningful way required the development of an internally standardized and highly reproducible PCR technique. The initial studies showed that lamina propria mononuclear cells from patients with inflammatory bowel disease contain a full repertoire of TCR-Vβ families. Quantitative RT-PCR showed relative reductions in TCR-$V\beta_2$ mRNA values, but normal levels for Vβ families 6, 7, and 14. The abnormality in TCR-$V\beta_2$ expression is taken as suggestive evidence that a particular antigen or superantigen participates in disease pathogenesis.

In a second study, Dr. Strober and his colleagues, particularly Drs. Murakawa and Kitani, are defining the role of the LAM-1 adhesion molecule in T cell and B cell function. Cells that express LAM-1 home to lymph nodes. It was postipulated that when a lymphocyte adheres to lymph node tissues through interaction with LAM-1, further activation of the lymphocyte might take place. To address this, biochemical studies were performed of the interaction of LAM-1 with other lymphocyte cell surface moieties. This seemed to be reasonable because attachment of LAM-1 antibodies to lymphocyte cell surface augmented CD3-mediated stimulation of T cells. By immunoprecipitation and gel electrophoresis, it was possible to show that LAM-1 aggregates with the CD3 surface complex.

Differentiation of B cells to synthesize IgA has been another long standing interest in this Section. In the past year two approaches have been taken to this problem. In the first, a molecular biological approach was used to assess the involvement of A-GLT in IgA switch differentiation. It was reasoned that inhibition of A-GLT transcription might impair IgA switch differentiation. IgA switch differentiation was induced to moderate levels using IL-4 and TGF-β. A-GLT transcription was inhibited by transfecting into B cells plasmids expressing oligonucleotides that were either sense, anti-sense, or non-sense relative to A-GLT transcription start site. Only the anti-sense construct should work specifically at inhibiting A-GLT transcription. The result was that IgA switch was significantly inhibited only cells transfected with the anti-sense oligonucleotide construct, but not the others, verifying a role for A-GLT in IgA switch. An additional part of these studies entails molecular analyses of the RAG genes. Peyer's patch B cells were found to specifically express RAG-2. The RAG-2 gene was cloned and sequenced.

A final series of studies engage a cooperative effect between Dr. Michael Sneller, a former fellow in the Section, and others of the Section's clinical associates. The goal is to define the biological bases for common variable immunodeficiency (CVI). In recent years, the group showed that nearly one third of patients with CVI have an unusual lymphocyte phenotype, in which there is an expansion of the class of CD8-positive T cells. These cells were shown to respond poorly to proliferative stimuli and to release abnormally low amounts of IL-2. On the other hand, these CD8-positive T cells produced increased amounts of IL-5 and gamma interferon. These also had increased cytotoxic suppressor activity. These data imply an ongoing level of T cell stimulation, possibly as result of a chronic viral infection. This possibility has been explored preliminarily by analyzing cultured CVI patient B cells by electron microscopy and by reverse transcriptase assays. Although neither yet shows evidence of a virus, there is more to be done.

Dr. Sneller is hoping to exploit the observed functional and phenotypic differences in CVI patients and patients with other immuno-deficiency diseases by the development of exploratory protocols for immunotherapy. In this regard, he is about the initiate a trial of gamma interferon for patients with the hyper-IgE syndrome. He has also undertaken exploratory cytokine and immunotherapeutic treatments of a young child with a novel lymphoperifitive disease characterized by a vast expansion of T cells lacking both CD4 and CD8 from their surfaces. Initial studies with high dose intravenous immunoglobulin, with recombinant alpha interferon, and with recombinant IL-2 were of little value. The child is currently on cyclosporin. It is desired that these types will expand into larger human models of primary immunodeficiency states.

1991-1992
Honors and Awards
Laboratory of Clinical Investigation, NIAID

During FY 92 several honors were bestowed on LCI staff members.

Dean D. Metcalfe
20th Annual Clemens von Pirquet Award from

Georgetown University

K.J. Kwon-Chung
Received $2,500 and a Gold Medal as part of th Sung-Ji

Academy Award from the Korean National Academy of

Science

Janet K. Dale
Director's Award from Dr. Healy

Stephen Straus
Elected to the Association of American Physicians

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00048-21 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. Frank - Chief - LCI, NIAID
C. Hammer - Senior Investigator - LCI, NIAID
M. Basta - Visiting Fellow - LCI, NIAID
P. Langlois, IRTA Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

L. Fries, Johns Hopkins School of Public Health, Baltimore, MD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI0057-19-LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic Studies on Pathogens Causing Cryptococcosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

K.J. Kwon-Chung, Research Microbiologist, Clinical Mycology Section, LCI, NIAID
Others: Ashok Varma, Microbiologist, Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic association of mating types and virulence for mice were found in *Cryptococcus neoformans*. A pair of congenic *Cryptococcus neoformans* var. *neoformans* strains, B-4476 (a) and B-4500 (α) that presumably only differ in mating type, was constructed. This pair and their progeny, five α type and five a type, were tested for virulence in mice. In the parent strains as well as the progeny, α type was clearly more virulent than a type. In addition, death tended to occur earlier among the mice infected with α strains than the mice that died by infection caused by a strains. All unstable URA 5 transformants (DNA mediated) of *C. neoformans* var. *neoformans* exhibited the presence of an autonomously replicating plasmid which had undergone significant sequence rearrangement. The autonomously replicating plasmids in the transformants were maintained in linear form and had acquired genomic sequences with homology to a sequence(s) on all the chromosomes. These results suggest mechanisms of plasmid maintenance in *C. neoformans* that are different from those reported for other yeasts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-A1 0058-18 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Chemotherapy of Herpesvirus Infection in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI, NIAID
J. Cohen - Senior Staff Fellow - LCI, NIAID
R. McKenzie - Medical Officer - LCI, NIAID
R. Kost - Medical Staff Fellow - LCI, NIAID
T. Heineman - Medical Staff Fellow - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID
P. Krause - Medical Staff Fellow - LCI, NIAID
J. Dale - Research Nurse - LCI, NIAID

COOPERATING UNITS *(if any)*

J. Rooney, Senior Staff Fellow, LOM, NIDR; A. Notkins, Chief, LOM, NIDR;
L. Stanberry, University of Cinncinnati

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.6	PROFESSIONAL: 3.6	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of the project are to characterize the pathogenesis, natural history and therapy of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. Our clinical emphasis has been on oral and genital herpes and zoster. Analysis of 10 years of suppressive acyclovir therapy in patients with frequently recurring herpes have shown the drug to remain effective and well-tolerated. We developed and continue to test a model system in which ultraviolet light induces recurrent oral or sacral HSV infections in humans. In placebo-controlled trials topical sunblockers and acyclovir were found to significantly prevent uv-induced reactivation of HSV infection. We are now studying topical capsaicin, a substance P depleting agent to determine whether it will prevent recurrent oral herpes. A placebo-controlled trial showed that oral acyclovir significantly reduces rates of HSV1 outbreaks in patients with frequent oral recurrences.

The major basic research thrust of our laboratory has been to define molecular aspects of HSV and VZV latency and pathogenesis. During the past year we completed one series of studies examining the nature and role of the latency-associated gene of HSV2. Mutant virus lacking only the promoter for this gene was shown to induce typical genital herpes in guinea pigs, but to have a markedly lower likelihood of reactivating. Specific VZV genes involved in latency and regulation are also examined. Genes 29 an 62 are expressed in human ganglia. The promoters, transcripts and regulation of these genes and VZV genes 4, 10, 28, 61 and 63 are being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-A1 0058-18 LCI
cont.

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Chemotherapy of Herpesvirus Infection in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H. Moriuchi - Visiting Fellow - LCI, NIAID
M. Moriuchi - Guest Worker - LCI, NIAID
B. Savarese - Research Nurse - LCI, NIAID
E. Grigg - Guest Research Nurse - LCI, NIAID
L. Perera - Senior Staff Fellow - LCI, NIAID
Y. Yi - Visiting Fellow, LCI, NIAID

COOPERATING UNITS *(if any)*

J. Rooney, Senior Staff Fellow, LOM, NIDR; A. Notkins, Chief, LOM, NIDR;
L. Stanberry, University of Cinncinnati

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.6	PROFESSIONAL: 3.6	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00154-17 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Immediate Events in Hypersensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Martha V. White, M.D.	Senior Staff Fellow	LCI/NIAID
	Yasushi Igarashi, M.D.	Special Volunteer	LCI/NIAID
	Ann Daniels	Biologist	LCI/NIAID

COOPERATING UNITS *(if any)*
James Baraniuk, M.D., Georgetown University
Julie Goff, Ph.D., Georgetown University (Contract #NO-1-AI-22665

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This broad based project has explored various expressions of allergy over the past 17 years, ranging from anaphylaxis to asthma and rhinitis. One of the areas we have focused upon is the histamine releasing factor released from human neutrophils (HRA-N). HRA-N causes histamine release without concomitant arachidonic acid secretion, suggesting that unique pathways are involved. Corticosteroids are useful in the treatment of allergic diseases and one of the mechanisms by which it works was found to involve the ability of corticosteriods to inhibit HRA-N induced histamine release. This observation extends earlier work on the effects of corticosteroids on mast cell reactivity.

Allergen challenge of the nasal mucosa in allergic individuals elicits an acute allergic reaction. We examined the effects of nedocromil on nasal provocation and found it to have no effect, suggesting that nedocromil may not be useful in the treatment of allergic rhinitis. The symptoms of allergic rhinitis are largely due to histamine-induced vascular permeability. We examined the distribution of histamine receptors in the nasal mucosa and found that H-1 receptors were localized to the vascular tissues exclusively. IL-4 is used in cancer chromotherapy but causes distressing nasal congestion, which often limits the use of IL-4. The congestion appears to be due to histamine release, perhaps due to increased susceptibility of nasal mast cells to HRA-N.

PHS 6040 (Rev 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00249-11 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. - Head, Mast Cell Physiology Section, LCI/NIAID

Others: JoAnn Mican, M.D. - Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

Clincial Pathology Department, NIH Clinical Center (Dr. William Travis)

Laboratory of Host Defense Mechanisms, NIAID (Dr. John Gallin)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.75	0.50	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with mastocytosis are more likely to develop significant liver pathology if they have category II disease (mastocytosis with an associated hematologic disorder) or category III disease (aggressive mastocytosis). Liver pathology includes mast cell hyperplasia and fibrosis, sometimes leading to portal hypertension. Patients with category I disease (indolent mastocytosis) are less likely to develop these problems. Liver pathology tends to correlate with laboratory parameters of liver disease. Liver-spleen scans were of value in assessing liver size, and in identifying other types of problems such as liver hemangiomas. Based on these findings routine liver biopsy is not recommended in the evaluation of mastocytosis. Liver biopsy should only be performed on patients with laboratory and scan evidence of significant liver disease.

- Preliminary studies are underway to assess cytokine profiles in mastocytosis patients, with emphasis on stem cell factor. Suction blisters are raised over urticaria pigmentosa lesions which contain collections of mast cells. Blister fluid is assayed for cytokines appearing in blister fluid over several hours. It is hoped that this study will identify an abnormal cytokine profile associated with mast cell hyperplasia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00275-10 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effect of PMN Oxidative Products on Membrane Receptor Expression and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. Gaither - Senior Investigator - LCI, NIAID
M. Frank - Chief - LCI, NIAID
Yannick Pilatte - Guest Researcher - LCI, NIAID
Evelyn Lin - Chemist - LCI, NIAID
Tiffani Durant - Laboratory Worker - LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.75	PROFESSIONAL: 2.0	OTHER: 0.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279-11 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael A. Kaliner, M.D. Head Allergic Diseases Section LCI/NIAID

COOPERATING UNITS *(if any)*

James H. Shelhamer, M.D., Carolea Logun, M.D., Critical Care Medicine, Clinical Center, NIH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosa have been developed in order to examine the controls of mucus secretion. Mucous glycoproteins were purified and monoclonal antibodies produced. Employing a monoclonal antibody directed at a mucous glycoprotein secreted from mucous cells of the submucosal glands, an Elisa assay for mucus was developed. This assay was employed along with measurement of serous cell products to compare glandular responses from human tissues both in vitro and in vivo. Neither beta adrenergic agonists or histamine caused secretions from either model while alpha adrenergic compounds and muscarinic agonists were active in both models.

We also compared the response of nasal mucosa in vitro to several neuropeptides and found that substance P and neurokinin A were moderately stimulatory for gland secretion.

PHS 6040 (Rev. 1/84)

U S GOVERNMENT PRINTING OFFICE 1991 0—864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00354-10 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Warren Strober, M.D.	Head, MIS Section	LCI, NIAID
	Rainer Duchmann, M.D.	Special Volunteer	Deutsche Forschungsgemeinschaft

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the past several years we have examined the hypothesis that the inflammatory bowel diseases, ulcerative colitis and Crohn's disease, is due most fundamentally to an abnormal immune response to one or more mucosal antigens that are always present in the mucosal environment. During this period we investigated this possibility by assessing TCR-Vβ family repertoire expression in T cells in the lamina propria (LPMC) and peripheral blood (PBMC) of IBD patients and control individuals. Our strategy for making such determinations was based on a quantitative RT-PCR technique that has been developed in this laboratory. This technique involves first the construction of internal mRNA standards having the same primer sites as the mRNA to be quantitated but having a different size from the latter and second the co-reverse transcription and amplification of the standard mRNA. The amount of "unknown" TCR-Vβ mRNA is determined in this method from an equivolence point at which standard and unknown are transcribed and amplified with equal efficiency. In our initial studies we found using a qualitative RT-PCR technique that LPMC mRNA of IBD patients contained a full repetoire of 20 TCR-Vβ families and that IBD is not associated with gross deletion or expansion of any family. In further studies with quantitative RT-PCR of four freqently expressed TCR-Vβ families, we found that relative TCR-Vβ expression in LPMC + PBMC was quite different, probably reflecting the somewhat different antigen-drive giving rise to these cell populations. More importantly, we found that mean mRNA values for TCR-Vβ_2, but not TCR-V$\beta_{6,7}$ and $_{14}$ were lower in IBD patients than in control individuals. These results indicate a selective abnormality of TCR-Vβ_2 expression in both forms of IBD. They are therefore consistent with the concept that a particular antigen or superantigen is important in disease pathogenesis.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00356-10 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Regulation of IgA Immunoglobulin Synthesis.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID

Other: Yoshio Wakatsuki, M.D., Visiting Fellow, MIS/LCI/NIAID
Rolf Ehrhardt, M.D., Visiting Fellow, MIS/LCI/NIAID
Milan Basta, M.D., Visiting Associate, MIS/LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda MD 20892-0010

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we are examining the various cellular and molecular processes that occur during the differentiation of IgA B cells, the main type of B cell occurring in the mucosal immune system. In one phase of this study we transfected CH12.LX B cells with plasmid vectors containing dexamethasone-inducible cassettes producing oligonucleotides that were either sense, anti-sense or non-sense to the α-GLT transcription start site (Iα start site), and then measured the effect of such transfection on IL-4/TGF-β-induced α-GLT levels and IgA switch differentiation. We found that cells transfected with anti-sense oligonucleotide-producing plasmids, but not cells transfected with sense or non-sense oligonucleotide-producing plasmids, exhibited reduced α-GLT levels, as well as decreased membrane IgA expression and IgA secretion. Thus, α-GLT appears to be necessary for successful IgA switch differentiation. In a second phase of the study, we measured the expression of RAG genes (recombination activation genes) in mucosal and system lymphoid tissues. Using RT-PCR, we found that B cells in murine Peyer's patches, but not in spleen or lymph nodes, express the RAG-2 gene, but not the RAG-1 gene. Spleen cells cultured in IL-4 alone also express RAG-2 mRNA; this, plus the fact that RAG-2 is expressed in Peyer's patch B cells suggests that the RAG-2 gene product is necessary for certain forms of isotype differentiation. Finally, we studied the activation requirements of post-switch sIgA-bearing B cells. In these studies we showed that whereas sIgM/sIgD-bearing B cells proliferate and produce Ig when stimulated with anti-IgD-dextran, both sIgG-bearing B cells from peripheral lymph nodes and sIgA-bearing B cells from Peter's patches respond with neither proliferation nor secretion (of IgG or IgA, respectively) when stimulated with anti-IgG-dextran or anti-IgA- dextran, respectively. In contrast, such cells do proliferate and secrete when stimulated by T cells. These studies show that isotype-switched B cells require a signal from T cells to undergo terminal differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00429-08 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Nasal Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
	Martha V. White, M.D.	Senior Staff Fellow	LCI/NIAID
	David B. Peden, M.D.	Medical Staff Fellow	LCI/NIAID
	Yasushi Igarashi, M.D.	Fogarty Visiting Fellow	LCI/NIAID
	Kimihiro Ohkubo, M.D.	Special Volunteer	LCI/NIAID
	Chul Hee Lee, M.D.	Fogarty Fellow	LCI/NIAID
	Bernard Mosimann, M.D.	Special Volunteer	LCI/NIAID
	Con't on page two		

COOPERATING UNITS *(if any)*
James H. Shelhamer, M.D., Critical Care Medicine Clinical Center, NIH
Robert Lebovics, M.D., NIDCD, NIH

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.3	5.8	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The nasal mucosa is the first internal surface to encounter aeroallergens, airborne pathogens, and airborne toxins. Analysis of nasal responses will likely provide insights into normal host defense mechanisms at the mucous membrane level. A guinea pig model of nasal responses was developed and was shown to resemble human cholinergic, histamine and neuropeptide responses. Guinea pigs were desensitized to capsaicin in order to allow examination of the contribution of sensory neuropeptides to nasal responses; histamine induced secretions were significantly reduced suggesting a role for nuropeptides in allergic reactions. The primary muscarinic receptor on human submucosal glands was determined to be of the M_3 type, both by receptor analysis and by functional analysis. M_3 mRNA was also localized to the glands. The primary enzymes involved in metabolizing the neuropeptides were characterized: neutral endopeptidase is found in the endothelium, epithelium and serous cells while angiotensin converting enzyme is in the interstitium. Proteases released form PMN might be capable of damaging the nasal epithelium. However, the serous cells were found to synthesize and secrete secretary leukoprotease inhibitor (SLPI). SLPI constitutes up to 3% of secreted protein and protects the nasal epthelium during infections. Rhinovirus is the commonest virus to cause upper respiratory infections (URI). URI's are universally experienced infections causing rhinorrhea. The source of rhinorrhea during the initial phase of a URI is the vascular bed due to increased vascular permeability. During the resolution phase of a URI, the source of secretions is glandular.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-364-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00429-08 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Nasal REsponses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Others:	Michael Goldrich, M.D.	Special Volunteer	LCI/NIAID
	Hiroshi Nagata, M.D.	Special Volunteer	LCI/NIAID
	Ronald Moss, M.D.	Medical Staff Fellow	LCI/NIAID
	Thomas Scott, M.D.	Medical Staff Fellow	LCI/NIAID
	Ann Daniel, M.D.	Biologist	LCI/NIAID

COOPERATING UNITS *(if any)*

James H. Shelhamer, M.D., Critical Care Medicine Clinical Center, NIH
Robert Lebovics, M.D., NIDCD, NIH

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.3	5.8	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z0 AI 00430-08 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Varicella-Zoster Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.I. Cohen - Senior Staff Fellow - LCI, NIAID
S. Straus - Senior Investigator - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID
R. Kost - Medical Staff Fellow - LCI, NIAID
T. Heineman - Medical Staff Fellow - LCI, NIAID
H. Moriuchi - Fogarty Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Varicella-zoster virus causes chickenpox on primary infection, and zoster during reactivation from viral latency. The goals of this project are to identify and determine the function of varicella-zoster virus (VZV) genes that are expressed during active infection and during latency in the body.

RNA transcripts corresponding to immediate-early and early VZV genes have been detected in human trigeminal ganglia using in situ hybridization and Northern analysis. At present, we mapping transcripts from these VZV genes and assaying their ability to regulate expression of other VZV genes by transient expression assays in vitro. VZV genes that are expressed during active infection and regulate viral gene expression are being studied. The transcriptional activation or repression domains of two of these regulatory proteins are being mapped. Cell lines have been constructed that stably express several of these VZV proteins and we are analyzing the effect of these proteins during infection of the cells with wild-type VZV or herpes simplex virus mutants. In addition cell lines are being constructed that secrete VZV glycoproteins which will be useful for evaluating the immune response to VZV and may be useful for generating subunit vaccines. Finally, we are constructing VZV mutants which are deleted for several of these regulatory genes or for genes encoding viral glycoproteins. Analysis of these viral mutants in cell culture and in animal models should provide further information on the role of individual VZV genes in active infection and during latency.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00 432 08 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of immune responses in humans and non-human primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID
Other: Yohko Murakawa, M.D., Visiting Associate, MIS/LCI/NIAID
Atsushi Kitani, M.D. Visiting Fellow, MIS/LCI/NIAID
Chingchai Wanidworanun, M.D., Visiting Associate, MIS/LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	3.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In these studies we are defining the role of the LAM-1 adhesion (MEL-14 lymph node homing receptor) molecule in T cell and B cell function. In preliminary studies, we determined that anti-LAM-1 antibodies can augment anti-CD3-induced proliferation of purified T cells. In further studies we immunoprecipitated surface radioiodinated T cell lysates with anti-CD3 and anti-LAM-1 mAb to determine if molecules in the TCR/CD3 complex associate with Leu 8 molecules. Although Leu 8 mAb immunoprecipitated only a single protein of approximately 80 kDa from T cell lysates treated with Nonidet P-40 under reducing condition, it coimmunoprecipitated additional proteins of 48, 42, 28, 24, and 22 kDa from T cell lysates treated with 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS). These additional proteins were identified as the α-, β-, γ-, δ-, and ε-chains of the TCR/CD3 complex by one-dimensional and two-dimensional diagonal SDS-PAGE. Densitometric scanning showed that, on average, 18% of the TCR/CD3 complex associates with LAM-1. In a final study, we showed by immunoblotting anlaysis using anti- peptide antibody that anti-LAM-1 mAb coimmunoprecipitates the chain of CD3. These results indicate that the human-lymph node homing receptor homologue (LAM-1) participates in the activation of T cells, probably via its association with the TCR/CD3 complex.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-A1 00469-07 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

COMPLEMENT: Studies in Viral Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robin McKenzie, M.D., LCI, NIAID

COOPERATING UNITS *(if any)*

Brian Murphy, M.D., LID, NIAID, Bernard Moss, M.D. LVD, NIAID
Michael Frank, M.D., Duke Univeristy

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two projects were undertaken to study the interaction of complement with viruses. The first project utilized a monoclonal antibody which caused complement-medicated neutralization of parainfluenza virus type 3. Since neither this antibody nor complement alone neutralized the virus, they provided a model to investigate the interaction between antibody and complement which was crucial for neutralization. Complement was bound to the virus without antibody, but only with antibody was the virus lysed. Results suggested that the antibody directed C4 binding to the area of the HN protein where further complement depostion caused virolysis.

A second project is the characterization of a major protein secreted by vaccinia virus. This protein, which is homologous to C4-binding protein, inhibits activation of human complement thereby potentiating the pathogenicity of vaccinia virus in the human host. Upon completion of these phases of work, the project was terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00470-07 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chronic Epstein Barr Virus Infection and Chronic Fatigue Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI, NIAID
J. Dale - Clinical Research Nurse - LCI, NIAID
E. Benson-Grigg - Guest Research Nurse - LCI, NIAID
R. McKenzie - Medical Officer - LCI, NIAID
J. Cohen - Senior Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

R. Johnson, J. Grafman (LMN, NINCDS), H. Rotbart (Univ. of Colorado), M. Demitrack (Univ. of Michigan), M. Altemus, P. Gold (NIMH), P. Peeke, D. Garcia, G. Chrousos (NICHD), W. Strober, M. Sneller (LCI, NIAID), Scott Fritz (PRI, FCRF)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to characterize severe chronic infections with Epstein Barr Virus (EBV) and to elucidate multiple aspects of the chronic fatigue syndrome which was, earlier, considered to be related to EBV infection. To date this research project has involved nearly 190 patients. Included are 7 patients who were diagnosed with severe chronic EBV infections on the basis of clinical, histological, molecular and serologic features. We continue to examine immunologic features of patients with severe chronic EBV-associated lymphoproliferation and explore treatments. Acyclovir, alpha and gamma interferons proved of little value, but immunosuppressive therapies are being used with good long-term results.

Detailed immunologic, neurologic, endocrinologic and psychologic studies are being conducted on selected patients with chronic fatigue. To date, we still have no consistent laboratory abnormality that permits a clear diagnosis of the chronic fatigue syndrome, however, we have been pursuing the basis and meaning of group abnormalities in neuropsychiatric, immune and endocrine systems. A series of studies of the pituitary-adrenal responsiveness to corticotropin releasing hormone and ACTH and of neuropeptide and catechol levels in spinal fluid suggest deficient central CRH release. Since CRH induces CNS arousal, these neuroendocrine findings suggest a new mechanism whereby the lethargy of Chronic Fatigue Syndrome patients may be explained. We are pursuing these observations in a new series of studies and a fresh patient cohort. Arginine-Vasopressin infusions are being given to stimulate and test the HPA axis. A placebo-controlled trial of hydrocortisone treatment has begun. It should permit us to test the hypothesis that corticosteroid deficit leads to symptoms. We also recognized discrete abnormalities in CFS patient lymphocyte phenotype and in vitro responsiveness to mitogens in patterns suggesting mild immune activation. There is a reduction in naive (CD4xCD45RA) T cells and an increase in memory (CD4xCD45RO) T cells bearing adhesion markers (LFA3, CD29, etc.). A vigorous, multidisciplinary approach to the syndrome continues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00495-06 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Biochemical Events in Mast Cell Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Robert J. Hohman, Ph.D.	Expert	LCI/NIAID
	Thomas Hultsch, M.D.	Fogarty Associate	LCI/NIAID
	Prescott Atkinson, M.D.	Medical Staff Fellow	LCI/NIAID
	Raynaldo A. Martin	Biologist	LCI/NIAID
	Susan A. Bardwell	Medical Student	

COOPERATING UNITS *(if any)*
Dr. Sue Goo Rhee, Lab. of Biochem., Sect. on Sig. Transduction, Bethesda, MD
Dr. Alam Weissman, NCI

LAB/BRANCH
Laboratory of Clincal Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biochemistry of IgE receptor-mediated activation of mast cells is being studied in two separate but complementary projects which are summarized below.

1) *Inhibition of IgE receptor-mediated exocytosis from mast cells by the immunosuppressive drugs cyclosporin A (CsA) and FK506.*

Immunosuppressive drugs such as CsA and FK506 are considered by many to specifically inhibit T cell functions; however, the same concentration of CsA that inhibits T cell receptor-mediated activation of T cells also inhibits IgE receptor-mediated degranulation of mast cells. These studies have been extended by studying the effect of FK506 and its structural analog, rapamycin, on IgE receptor-mediated degranulation of mast cells. The intracellular target for rapamycin and FK506 (FKBP) is distinct from the intracellular target for CsA (cyclophilin). Like CsA, FK506 inhibits degranulation of mast cells without effecting any other IgE receptor-mediated function; however, FK506 is 100 times more potent than CsA. The IC_{50} for inhibition is 200 nM and 2 nM for CsA and FK506, respectively. Rapamycin and 506BD both compete with FK506 for binding to FKBP. Pretreatment of mast cells with either rapamycin or 506BD prevents the inhibition by FK506. Thus, it appears as though a FKBP-FK506 complex as well as a cyclophilin-CsA complex inhibits receptor-mediated activation of mast cells.

2) *Activation of membrane-associated phospholipase C by aggregation of IgE receptors*

Currently, little is known concerning the mechanism of activation of PLC and, in fact, it is not even known which of the PLC isozymes present in mast cells is involved in receptor-mediated PI hydrolysis. A major obstacle to these studies is the lack of a cell-free system in which this activation can be studied. We have established a system in which aggregation of IgE receptors results in activation of membrane-associated PLC. Rat basophilic leukemia (RBL) cells are activated by aggregation of the IgE receptors. The PLC activity in membranes isolated from activated cells is increased by 150% above membranes from control cells. This system will be used to identify which PLC isozyme is activated and the mechanism of this activation.

U S GOVERNMENT PRINTING OFFICE 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00496-06 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interaction Between the Human Immunodeficiency Virus (HIV) and Herpesvirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: S. Straus - Senior Investigator - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*
D. Margolis, (University of Massachusetts)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying the interactions between HSV and HIV genes at a molecular level. In the first project we are examining the mechanism by which HSV-1 activates HIV RNA transcription. The bulk of HSV activation is dependant on the integrity of Spl and NF-kB enhancers in the HIV LTR. HSV also activates the LTR by inducing the expression of a cellular protein that binds near the HIV transcriptional start site. Competition experiments proved the binding specificity of the protein and uV-cross linking studies identified a novel 45kD protein. We are currently attempting to purify the protein, termed LBP-2.

In the second series of studies we showed that the HIV tat transactivates the HSV-1 latency-associated gene. Transient expression assays showed marked upregulation of LAT promoter but not other classes of HSV-1 promoters. Site-directed alterations in the LAT promoter sequence are being used to define that tat target.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00513-05 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. - Head, Mast Cell Physiology Section, LCI/NIAID

Others:
Parris Burd, Ph.D. - Senior Staff Fellow, LCI/NIAID
JoAnn Mican, M.D. - Medical Staff Fellow, LCI/NIAID
Menachem Rottem, M.D. - Medical Staff Fellow, LCI/NIAID
Peter Bianchine, M.D. - Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

Beth Israel Hospital (Galli)
Georgetown University (Goff)
Amgen (Zsebo)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.00	2.75	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mast cells were examined in order to evaluate the biologic characteristics, function, and differentiation of mast cell precursors;to evaluate the role of adhesion receptors in mast cell biology; and to understand mast cell cytokines.

Mast Cell Precursor Biology

Mast cell precursors from murine bone marrow have an obligatory requirement for IL-3 early in maturation, after which they mature into the mucosal phenotype in IL-3 alone, or toward the connective tissue phenotype in c-kit ligand (stem cell factor). Fc_eRI+ mast cell precursors are also positive for Fc_gRII/III. Acquisition of Fc_eRI is associated with a diminished proliferative capacity suggesting it is a marker of terminal differentiation.

Human $CD34^+$ bone marrow derived cells give rise to mast cells on c-kit-negative fibroblasts only when these fibroblasts have been tranfected to produce membrane bound c-kit ligand with or without producing the soluble form of c-kit ligand.

Mast Cell Adhesion Receptors

Murine mast cells spontaneously adhere to vitronectin through an $alpha_v$ $beta_x$ integrin in a calcium dependent process. When mast cells adhere to vitronectin in the presence of IL-3, their proliferative rate increases. This is the first evidence of an adhesion receptor mediated alteration in mast cell behavior. Murine mast cells express ICAM-1 and VCAM-1 and display heterotypic aggregation with murine lymphocytes.

Mast Cell Cytokines

IL-6 is produced in mouse skin following IgE-mediated degranulation demonstrating that IL-6 of one of the mediators of inflammation that follows allergic reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00514-05 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diagnosis and Treatment of Adverse Reactions to Foods and Additives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Dean D. Metcalfe, M.D. - Head, Mast Cell Physiology Section, LCI/NIAID

Others: None

COOPERATING UNITS *(if any)*
Indian Institute of Science, Bangalore, India (P.V. Subba Rao)
Mucosal Immunity Section, LCI/NIAID (Jaffe)
Clinical Neuroscience Branch/ NIMH (Martin)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mast Cell Physiology Section

INSTITUTE AND LOCATION
NIAID, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0.00

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Allergic eosinophilic gastroenteritis (AEG) is a disorder chracterized by elevated IgE, specific IgE to food antigens, and eosinophilia of tissue and peripheral blood. IL-5 synthesis by $CD4^+$ and $CD8^+$ T lymphocytes from patients with AEG occurs in the absence of mitogen. IL-4 and IL-5 production from AEG lymphocytes was markedly elevated with mitogenic stimulation. GM-CSF and gamma-IFN synthesis was normal. Gamma-IFN mRNA was not detected by RT-PCR in mucosal biopsies obtained from patients with AEG, but was seen in normal biopsies. These observations document a cytokine imbalance which appears responsible for the induction of IgE and eosinophilia observed in this disorder.

Anti-idiotypic antibodies (Ab-2) are the mirror images of idiotypic antibodies (Ab-1). Murine Ab-2 raised by immunizing mice with Ab-1 specific for Parthenium hysterophorus functioned as surrogate allergens in in vitro diagnostic tests. Such surrogate allergens may be of value in allergen standardization and for in vitro diagnosis particularly when it is difficult to obtain and purify specific allergens in large quantities.

Tryptic digestion of the major shrimp allergen (Pen i I) yielded large peptides, one which retained activity. Amino acid sequencing on these peptides has been completed, and revealed 80% homology to Tropomyosin from the fruit fly, Drosophila melanogaster.

Monosodium glutamate to date has not had an effect on bronchial hyperreactivity in asthmatics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 0000548-04

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention of Genital Herpes Simplex Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI, NIAID
R. McKenzie - Medical Officer - LCI, NIAID
B. Savarese - Research Nurse - LCI, NIAID
R. Kost - Medical Staff Fellow - LCI, NIAID
T. Heineman - Medical Staff Fellow - LCI, NIAID
P. Krause - Medical Staff Fellow - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

R.L. Burke, M. Tigges, C. Dekker, (Chiron, Inc., Emeryville, CA)
L. Corey, (University of Washington, Seattle)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Having completed over a decade of work on antiviral treatment of genital herpes, we turned to studies of disease prevention. We completed an open 2 year phase 1 assessment of a new recombinant HSV-2 glycoprotein D vaccine in alum in 24 adults, some with and without prior HSV-1 and/or 2 infection. Vaccinations of 30 ug or 100 ug were given at 0, 1, 2, and 12 months and were associated with minimal local or systemic reactions. The vaccine induced excellent primary antibody in cellular immune responses and augmented preexisting humoral and cellular responses signicantly. We studied antibody and T cells from previously uninfected subjects and showed that the gD2 epitopes that the antibody and cells recognize correspond to those in genital herpes patients. On the basis of these excellent responses we enrolled 40 patients with recurrent gential herpes into a placebo-controlled vaccine trial in collaboration with the University of Washington. The goal is to determine whether boosted immunity leads to less frequent recurrences. The study was recently completed and the data are being examined. We have also begun to plan for further studies using gD2 and gB2 in the presence of potent new aadjuvant MF59.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00589-03

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification of Complement Resistance Determinants in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D. Senior Staff Fellow, NIAID, LCI
Suzanne Wenderoth, B.S., Biologist, NIAID, LCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

LCI/NIAID

SECTION

Bacterial Pathogensis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Complement resistance is an important virulence factor in the pathogenesis of bacterial gram negative infections. A more complete understanding of complement resistance determinants may lead to improved prevention or treatment of infections caused by these organisms. We have used transposon mutagenesis to randomly mutate a clinical isolate of *Escherichia coli* that is highly serum resistant. Isogenic mutants have been detected that have an increased sensitivity to serum. Of these, one group is capsule minus.We have defined that at least three physical loci are involved in capsule production and they are linked. One of the capsule genes disrupted has DNA homology to the Neisseria capsular transport gene. Capsule also protects against killing by neutrophils, but not the bactericidal effects of the defensins. In a systemic mouse model of infection capsule minus strains cause significantly less mortality. Therefore, these results strongly suggest that capsule is a major virulence factor. Studies on the regulation of capsule synthesis demonstrate increased gene transcription as growth temperature increases from 22°C to 42°C. Other isogenic mutants, however, are capsule positive but serum sensitive. This demonstrates that bacterial serum resistance is multi-factorial process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00590-03 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of Primary Immunodeficiency Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael C. Sneller, M.D.	Medical Officer	LCI, NIAID
Others:			
	Warren Strober, M.D.	Chief, Mucosal Immunity Sect.	LCI, NIAID
	Eli Eisenstein, M.D.	Clinical Associate	LCI, NIAID
	Jon S. Jaffe, M.D.	Clinical Associate	LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to further define the immunopathogeneic mechanisms responsible for the clinical syndrome known as common variable immunodeficiency (CVI). During the past year we extended our studies on the functional properties of the expanded population of CD8 T cells seen in a subgroup of patients with CVI. We demonstrated that these cells exhibit a defect in proliferation and IL-2 production. However, these CD8 T cells showed increased production of IL-5 and gamma-interferon. In addition, we found that these T cells were fully functional as cytotoxic T lymphocytes in anti-CD3 mediated cytotoxicity assays and exhibited increased suppressor activity for B cell immunoglobulin production. Taken together, this phenotypic and functional profile suggests that the expanded CD8 T cell subpopulation in this group of CVI patients contain highly differentiated (effector) T cells which are "anergic" from a proliferation and IL-2 production standpoint, but which nevertheless are immunoreactive from the point of view of gamma-interferon/IL-5 production and cytotoxic/suppressor activity. These findings raise the possibility that the CD8 T cells in this group of CVI patients are "foot prints" of a chronic viral infection which is the basic cause of the disease.

In a separate line of investigation, we been able, for the first time, to propagate nontransformed B cell lines from CVI patients, and show that these cells retain an abnormal phenotype in that they produce reduced amounts of immunoglobulins *in vitro* when compared to lines established from normal donors. The addition of a variety of lymphokines failed to normalize immunoglobulin production by CVI B cell lines. Electron microscopy and reverse transcriptase assays performed on the patient B cell lines failed to provide evidence of viral infection. Preliminary molecular studies performed on these cell lines suggest that the B cell defect in CVI may relate in part to a failure of immunoglobulin isotype switch. The ability to obtain large numbers of nontransfomed CVI B cells should greatly facilitate studies into the biochemical and molecular mechanisms of the CVI B cell defect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00595-01 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification, Characterization and Function of Proteins of the Complement System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. Hammer - Senior Investigator - LCI, NIAID
M. Frank - Chief - LCI, NIAID
T. Leto - Senior Staff Fellow - LCI, NIAID
T. Russo, Senior Staff Fellow - LCI, NIAID
R. Jacobs - Medical Staff Fellow - LCI, NIAID
G. Kerr, Visiting Fellow - LCI, NIAID
G. Linton - Medical Technologist - LCI, NIAID
L. Renfer - Chemist - LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.3	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00607-02 LCI

PERIOD COVERED

July 3, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cytokine Gene Expression in Mast Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Parris Burd, Ph.D. - Senior Staff Fellow, LCI/NIAID

Other: Dean D. Metcalfe, M.D. - Head, Mast Cell Physiology Section, LCI/NIAID
Chad Oh, M.D. - Medical Staff Fellow, LCI/NIAID
JoAnn Mican, M.D. - Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

LIR/NIAID (Ehrenreich)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.75	2.25	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Northern blot analysis shows that TCA3 is expressed after activation with either PMA/ionophore or high affinity IgE cross-linking in C57 cells. mRNA of TCA3 is induced within 30 min to 1 hour, peaks at 2 to 3 hours, and disappears at 4 to 6 hours. Half-life of mRNA determined by adding actinomycin D is approximately 2 hours. These results suggest the TCA3 is regulated transcriptionally. Nuclear run-on assays show that the rate of TCA3 mRNA transcription is increased after activation consistent with transcriptional regulation. CAT assay show no detectable promotor activity in TCA3 promotor CAT constructs up to 1.3 kb upstream TATA, while HIV-CAT/TATA constructs showed constitutive expression with 3- to 5-fold induction with PMA.

Primary bone marrow mast cells as well as various mast cell lines contain and secrete ET-1. ET-1 secretion is not directly related to mast cell degranulation, but rather synthesized and secreted hours later. Mast cells express a single class of high affinity ETA receptors resembling the ET receptor. Stimulation of mast cell ET-1 receptors does not provoke histamine release or result in a mitogenic response of bone marrow derived cultured mast cells.

We examined the effect of thrombin on astrocytic endothelins and found that endothelin-1 is released into the culture fluid in response to thrombin treatment. This increased production of endothelin-1 is not accompanied by an increase in steady state levels of endothelin-1 mRNA as assessed by reverse transcriptase polymerase chain reaction, even though thrombin stimulation leads to increased inositolphospholipid turnover and activation of the nuclear factor AP1. Thus, astocytic production of endothelin-1 may be mainly post-transcriptionally regulated in response to thrombin stimulation. In addition, two endothelin receptor genes (ET_A and ET_B) were found to be transcribed simultaneously in primary astrocyte cultures, and both thrombin and endothelin-1 stimulation result in a temporary decrease in ET_A mRNA. These studies suggest a role for thrombin in the regulation of brain perfusion through endothelin-1 expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z0 AI00621-02 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Transformation by Epstein-Barr Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.I. Cohen, Senior Investigator - LCI, NIAID

COOPERATING UNITS *(if any)*

D. Mosier, Medical Biology Institute

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epstein Barr virus (EBV) nuclear protein 2 (EBNA-2) is essential for B cell transformation by the virus. The goal of this project is to determine the mechanism of B cell transformation by EBV.

Inoculation of EBV-transformed lymphocytes into immunodeficient SCID mice cells results in the formation of EBV-containing B cell tumors of human origin. We constructed cell lines transformed by several EBV mutants which were isogenic, except for different deletions in their EBV nuclear protein 2 (EBNA-2) gene. The time to tumor formation for cell lines containing a given mutant correlated with the ability of the mutant to transform B cells in vitro and with the ability of the mutant EBNA-2 gene to transactivate the EBV latent membrane protein in transient expression assays. These data indicate that EBNA-2 plays a critical role for B cell tumor growth in SCID mice and for lymphocyte transformation in vitro.

EBV EBNA-2 transactivates expression of EBV and B cell genes and we have shown that EBNA-2 contains a 14 amino acid domain that directly activates transcription. Mutation of a single amino acid in this domain completely abolishes the ability to activate transcription and transform B cells in vitro. Substitution of the transcriptional activation domain of EBNA-2 with the activation domain of herpes simplex virus VP16 resulted in a chimeric virus that was able to transform B cells in vitro and transactivate expression of EBV and B cell genes. Thus, EBNA-2 and VP16 activate transcription by similar mechanisms and transcriptional activation is essential for EBV-induced B cell transformation in vitro and in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00617-02-LCI

PERIOD COVERED

October 1, 1991 - September 31, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Capsule Synthesis in a Pathogenic Escherichia coli Isolate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D., Senior Staff Fellow, NIAID, LCI
Suzane Wenderoth, B.S., Biologist, NIAID, LCI

COOPERATING UNITS *(if any)*

Susan Grottesman, Ph.D., Senior Investigator, NCI, LMP

LAB/BRANCH

LCI

SECTION

Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0,5	PROFESSIONAL: 0.25	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have determined that the capsular polysaccharide of a K54 clinical isolate of *Escherichia coli* is an important virulence factor. Capsular antigens in *E. coli* can be divided into two groups on the basis of microbiologic, physical and chemical characteristics. Group 1 capsules are associated with non-pathogens whereas, group 2 capsules are present in pathogenic extra-intestinal isolates. An understanding of group 2 capsular regulation may lead to novel therapeutic approaches, therefore we have undertaken studies in this area. Group 1 capsule regulators have been identified and cloned. We have examined by complementation, gene disruption and gene fusion experiments what effect they had on the group 2 capsule of our K54 clinical isolate.

Genes for both group 1 & 2 capsular polysaccharide are present and are non-allelic. The group 1 positive regulators RcsA & B stimulate group 1 capsule production but apparently not group 2. A group 1 negative regulator, lon protease, either directly or indirectly decreases group 2 gene transcription. This decrease in group 2 gene transcription is associated with an increased serum sensitivity. This is consistent with the protective effect of group 2 capsule against the bactericidal effects of serum. We are presently in the process of identifying and cloning the group 2 regulators genes which appear to be, at least in part, separate from the group 1 capsular regulatory genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00618-01 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Acquisition of an Increased Resistance to Host Defenses In Vivo

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. Russo - Senior Staff Fellow - LCI, NIAID
S. Wenderoth - Technician - LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section/Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.25	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z0 AI 00650-01 LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Chronic Hepatitis B Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator - LCI, NIAID
R. McKenzie - Medical Officer - LCI, NIAID
B. Savarese - Research Nurse - LCI, NIAID
R. Kost - Medical Staff Fellow - LCI, NIAID
T. Heineman - Medical Staff Fellow - LCI, NIAID
J. Meier - Medical Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

J. Hoofnagle, A. DiBisceglie (NIDDK), K. Smiles (Oclassen Pharmaceuticals, San Francisco, CA), L. Corey (University of Washington, Seattle)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been exploring the potential antiviral efficacy of a class of fluoropyrimidine nucleoside analogues. Initially we explored the drug FIAC for inibition of CMV infection in AIDS patients. Antiviral activity was not seen at tolerated doses and studies turned to this drug's metabolic derivative, FIAU. Studies showed intolerance at high doses but excellent tolerance and profound inhibitory activity at lower doses against hepatitis B virus (HBV). The initial studies involved HIV positive patients with co-existing chronic HBV infection. Profound and even permanent reductions in circulating HBV DNA and antigens was achieved with oral FIAU at daily doses of 0.1, 0.5, or 1 mg/kg for 14 days. Recently, we turned to a broader dose modification study in normal patients with chronic HBV infection. Studies are comparing antiviral efficacy at .05, 0.1, 0.25 and 0.5 mg/kg per day for 28 days. Initial results suggest profound inhibition of HBV infection at the lowest doses and in the absence of any detectible side effects. In the coming year, these studies will be extended to exploit these remarkable results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO AI00651-01 LCI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Recurrent Urinary Tract Infections In Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D., Senior Staff Fellow, NIAID, LCI
Suzane Wenderoth, B.S., Biologist, NIAID, LCI

COOPERATING UNITS *(if any)*
Dr. Walter Stamm, Seattle, WA

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Pathogensis Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

The most common site for extra-intestinal *Escherichia coli* infection is the urinary tract. There are a number of risk groups for this site of infection, one of which being young women in their sexually active years. Infection in this group accounts for 6-7 million office visits annually in the United States. *Escherichia coli* isolates are responsible for greater than 80-90% of these episodes. About 250,000 of these infections progress to acute pyelonephritis. In approximately 20% of these women frequent recurrences of infection (3-6/annum) follow the first episode. The morbidity, time lost from work and cost of this infectious syndrome is considerable. An improved understanding of the pathogenesis of this disease would be of significant importance. It is believed that the vast majority of recurrent UTIs are episodes of reinfection with different strains of *E. coli* rather than persistance of a single strain.We are employing pulse field gel electrophoresis to generates DNA "fingerprints" of bacterial strains isolated from women with recurrent UTIs. This will allow use to definitively establish how frequently a single strain versus different strains are responsible for this pattern of infection.

Our studies to date on isolates from eight women suggests that infection from a single strain may occur more frequently than is presently suspected. If a single strain is responsible for the majority of recurrent UTIs our therapeutic approach in this subset of women will be altered. Several possibilities could account for the above findings. We plan to genotype serially collect isolates from a larger number of women with recurrent UTIs. This will confirm the above findings We also plan to genotype fecal isolates from these women that were collected in between and during infectious episodes. This data should clarify whether the responsible strain was persisting within the gastro-intestinal tract or within the urinary tract.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00654-01-LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics, Cell Biology and Biochemistry of Candida albicans Maltase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John Bennett, M.D., Head, Clinical Mycology Section, LCI, NIAID
Others: Antonia Geber, M.D., Medical Staff Fellow, CMS, LCI, NIAID
Peter Williamson, M.D., Ph.D, Medical Staff Fellow, CMS, LCI, NIAID
K.J. Kwon Chung, Ph.D., Senior Scientist, CMS, LCI, NIAID

COOPERATING UNITS *(if any)*

Curtis Braun, Ph.D. and Stephen G. Withers, Ph.D., Dept. of Chemistry, University of British Columbia, Vancouver, BC, Canada

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.7	PROFESSIONAL: 1.5	OTHER: 2.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Metabolism of sucrose and maltose by C. albicans was found to occur by intracellular transport and hydrolysis by maltase, an alpha-glucosidase. Maltase is produced as a less active proenzyme which is cleaved at the C terminal end to produce a more active enzyme. Maltase is coded for by the structural gene CAMAL2, which has ben cloned from both cDNA and genomic DNA. Disruption of CAMAL2 is in progress in order to determine the function of CAMAL2 in virulence and saccharide metabolism. Transcription of CAMAL2 has been found to be glucose repressible. We have determined the upstream nucleotide sequences and will identify activation sites by several strategies. We have also demonstrated existence of a sucrose/maltose permease and are cloning the gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00655-01-LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on pathogenic fungi

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John Bennett, M.D., Head, Clinical Mycology Section, LCI, NIAID
Others: Peter Williamson, M.D., Ph.D., Medical Staff Fellow, LCI, NIAID
K.J. Kwon-Chung, Ph.D., Senior Investigator, LCI, NIAID

COOPERATING UNITS *(if any)*

John Inman, Ph.D, LI, NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diphenol oxidase from Cryptococcus neoformans has been purified and N-terminal amino acid sequences determined from cyanogen bromide digests. These sequences have been used to construct oligonucleotide probes for cloning the enzyme. The enzyme is found free or bound to the cell wall, depending upon growth phase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00656-01-LCI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cryptococcal vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John Bennett, M.D., Head, Clinical Mycology Section, LCI, NIAID
Others: Peter Williamson, M.D., Ph.D., Medical Staff Fellow, CMS, LCI, NIAID

COOPERATING UNITS *(if any)*

John B. Robbins, M.D., Rachel Schneerson, M.D., Sarvamangala Devi, Ph.D., NICHD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A vaccine composed of cryptococcal capsular polysaccharide (glucuronoxylomannan, GXM) covalently linked to tetanus toxoid was well tolerated and immunogenic in 25 normal volunteers given 25 mcg of polysaccharide and 25 given 50 mcg. There was no significant difference in the response between the two doses. Repeating the 25 or 50 mcg dose at 6 weeks did not significantly increase either the IgG or IgM antibody concentration. In mice, this vaccine conferred 70-80% protection against infection in mice pretreated either with the vaccine or serum from mice immunized with the vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00657-01-LCI

PERIOD COVERED

October 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic studies on Candida species.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

K.J. Kwon-Chung, Ph.D., Research Microbiologist, Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS *(if any)*

Brian Wickes, University of California, San Francisco
P.F. Lehmann, Medical College of Ohio, Toledo, Ohio

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three sets of medically important yeasts, *Candida albicans*, *C. tropicalis*, and *C. krusei* were compared with their putative synonyms (*C. langeronii* and *C. clausenii*; *C. paratropicalis*; *Itsatchenkia orientalis*) to determine if these synonyms are genetically distinguishable from each other. Molecular techniques used to accomplish this goal included pulsed-field electrophoresis and hybridization to species-specific probes. This study did not identify any distinguishing characteristics between synonyms, thus supporting the current taxonomic treatment of these pathogens. Diverse karyotypes were found for *Kluyveromyces marxianus* var. *marxianus* which is the sexual state of *Candida kefyr*. Two distinct karyotypes were discernable among the strains isolated from various sources. The enzyme profiles, however, showed great similarity and the majority of enzyme patterns did not vary. The enzyme profile was stable over the maintenance of the cultures while the karyotype was unstable due to the frequent chromosomal rearrangement.

Laboratory of Cellular and Molecular Immunology
1992 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Cellular and Molecular Immunology
October 1, 1991 through September 30, 1992

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up *in vitro* analogs in tissue culture, and determine the molecular basis for the phenomena. The laboratory employs techniques from the disciplines of cellular immunology, molecular biology, and protein biochemistry.

During its sixth year, the laboratory has pursued four major areas of research: 1) the molecular nature of costimulation, 2) the meaning of a half maximal response for interleukin 2 production, 3) T cell receptor αβ lineage commitment and the affinity model for repertoire selection in thymic development and 4) fetal thymic stem cells, T cell life span, T cell recirculation and tolerance to tissue specific antigens.

T Cell Activation

Studies of T cell activation of normal murine T cell clones of the T_H1 type have shown that two signals are required to stimulate the cells to make interleukin-2 (IL-2) and divide. One signal is given through the antigen-specific receptor that is uniquely expressed on each clone. The other signal is called costimulation and is delivered through a different receptor. Our major objective during the past year was to characterize the costimulatory signal. Other laboratories had identified the B7/BB1 molecule as the inducible ligand on the APC and CD28 and CTLA-4 on the T cell as potential receptors for costimulation in the production of interleukin-2 (IL-2). We explored the role of these molecules in a new assay for costimulation, the induction of competence to respond to interleukin-4 (IL-4). Murine T_H1 clones do not respond directly to IL-4. They first must be activated by stimulating them with antigens and APC. After 24 hr. the activated T cells then proliferate in response to IL-4. We demonstrated that costimulation is required during the induction phase in order to make the clones competent to proliferate in response to IL-4. Although supplying costimulation also led to the production of IL-2, stimulation with this lymphokine was not the only signal required to gain competence, because addition of IL-2 to a T cell receptor occupancy signal only allowed the cells to become partially competent to respond to IL-4. We concluded from these experiments that the costimulatory signal contributes directly to the attainment of full competence to respond to IL-4. To explore the molecules involved in this costimulation, we used a recombinant fusion protein between CTLA4 and a human Ig immunoglobulin constant region. This molecule reacts with B7/BB1 expressed on activated antigen-presenting cells. We showed that CTLA4Ig could inhibit proliferation and IL-2 production of both T_H1 and T_H0 murine clones but not IL-4 production by the latter. In addition it inhibited the ability of

T_H1 clones to gain competence to respond to IL-4. We concluded from these experiments that B7/BB1 is the critical ligand involved in costimulation for both IL-2 production and the gaining of competence to respond to IL-4.

(S. Umlauf, K. Brorson, S. Kitagawa, L. Chiodetti, and R. H. Schwartz, LCMI, NIAID; P. Linsley, Bristol-Meyers Squibb)

T Cell Subsets

When a population of T cells divides in response to an antigenic stimulus a range of concentrations of the antigen is required to go from no cells dividing to all the cells dividing. At the concentration required to achieve one-half of the maximal response, it has been demonstrated that one-half of the cells are dividing. The production of lymphokines by T cells shows a similar dose-response curve to antigen. However, the interpretation of the half-maximal response is not necessarily the same. Either all the cells are producing only one-half of their maximal potential, or one half of the cells are producing at their full potential while the other half are producing nothing. To decide between these two alternatives we examined the production of interleukin 2 by single T cell clones at different concentrations of antigen. The response appeared to be quantal (all or none) but two different levels of response were observed, one approximately 3-4 times the other. Furthermore, the dose response curve was biphasic with a decrease in the number of cells in each group at higher (>30nM) antigen concentrations. Our interpretation of these results is that induction of transcription of the IL-2 gene is what is quantal. Thus, the group of cells giving the low level of IL-2 production had only one chromosome being transcribed, whereas the group giving the higher level of IL-2 production was transcribing both chromosomes. The fact that all the cells were not found in the high positive group at large antigen concentrations possibly reflects a negative feedback regulation on IL-2 transcription. Experiments are currently in progress to test these two hypotheses.

(B. Beverly, A. Bendelac, D. Cassell, and R. H. Schwartz, LCMI, NIAID; D. Alling, NCI, NIAID)

T Cell Differentiation

During T cell development, thymocytes that are able to recognize self MHC are signalled to mature (positive selection), whereas those capable of self reactivity are selected to die (negative selection). Our studies deal with the paradox of how signaling resulting from a specific TCR-MHC interaction can determine the positive or negative selection events. Several investigations involving TCR transgneic mice revealed that distinct thymic antigen presenting cells are probably not responsible for these alternate forms of selection. Instead, they suggest that it is the affinity of the TCR-MHC interaction that controls the developmental outcome. Increasing the coreceptor expression (through a CD8 transgene) is able to enhance TCR recognition of MHC, and alter net selection effects from positive to negative. Likewise, enhancing MHC expression by increasing MHC gene dosage can alter developmental fate. Thymic selection can, in some cases, be determined also by tissue-specific peptides presented only on thymic epithelium. The mechanism responsible for commitment to the αβ or γδ lineage has also been approached using TCR transgenic mice. If TCR-β transcriptional regulatory elements are used to direct expression of the TCRαβ transgenes, γδ T cells do not appear. Instead, there appears a $CD4^-8^-$ cell bearing the TCR-αβ transgenic receptor, but with the developmental, phenotypic, and functional properties of γδ T cells. These results suggest that T cell functions may be associated with a specific lineage, irrespective of TCR usage. These findings have led to an investigation of the role of TCR transcriptional regulatory elements in lineage development. Finally, our previous studies indicated that the thymus is able to induce tolerance to self antigens not only by deletional mechanisms but also by clonal inactivation (anergy). Transfer of the nonresponsive cells into hosts that lack the self antigen or into in vitro culture results in a reversal of the nonresponsive state, indicating that antigen is required to maintain this nondeletional form of in vivo tolerance.

(F. Ramsdell, C. Pavlovich, and B. J. Fowlkes, LCMI, NIAID; E. Robey, Department of Microbiology and Immunology, University of California at Berkeley)

T Cell Tolerance and Memory

A stem cell has two properties that characterize it. One is the ability to give rise to all progenitors. The other is the ability for self renewal. Until now no one has identified a stem cell in the thymus. This year we showed that transfer of 14 day fetal thymus under the kidney capsule of SCID mice resulted in continued production of T cells for at least six months. Since the SCID bone marrow cannot provide productive stem cells to seed to the thymus, we conclude that the early fetal thymic tissue must contain a stem cell. The failure of others to observe this could have resulted from the age of the transferred thymus graft, the nature of the recipient mouse or the in vitro handling of the tissue. In a separate piece of work it was demonstrated that interleukin 7 arrests the differentiation of T cell receptor αβ-bearing cells (TCRαβ) and not the development of T cell receptor γδ-bearing cells (TCRγδ). This effect was reversible.

Previous work had demonstrated that virgin T cells capable of recognizing the male-specific antigen survive for only 5 months after adult thymectomy. This year we completed the study by demonstrating that T cells from mice primed to the antigen before thymectomy (memory cells) can still respond at 14 months. Thus, memory cells persist in the presence of antigen for much longer than virgin cells. Interestingly, a number of the memory cells had reverted to a virgin phenotype as defined by cell surface markers such as MEL14. Thus, these markers more likely reflect an activation state of the T cell rather than a signature for a memory T cell. Such activated cells have been shown to recirculate through the thymus, but in small amounts in experiments involving acute transfer of a bolus of mature T cells. In experiments we have carried out, by grafting two sets of congenic fetal thymus grafts into normal and SCID recipients, we found that such T cells can make up as much as 10% of mature thymocytes. Phenotypically the re-circulating population consists largely of activated cells with a slightly higher proportion of TCRγδ cells than found in the spleen or lymph node. Finally, last year we demonstrated that thymic epithelium grafted into allogeneic hosts tolerizes for thymic epithelial antigens but not to spleen or skin from the same allogeneic donor. We postulated that tolerance induction was tissue specific involving recognition of only those MHC-peptide complexes found in the organ. This year we extended our observations to syngeneic combinations and showed that thymic epithelium does not tolerize to skin even when the host and donor MHC is the same. Hence skin cells have an antigenic profile (MAP:MHC antigen profile) which is distinct from thymic epithelium. Interestingly, the grafted mice did not reject skin from Nu/+ littermates suggesting that there exists a peripheral tolerance mechanism for skin-specific antigens.

(A. Bonomo, G. Hisatake, A. Bendelac, and P.C.E. Matzinger, LCMI, NIAID)

Administrative, Organizational, and Other Changes

During the past year Drs. Satoru Kitagawa and Menelaus Manoussakis joined the laboratory and Drs. Adriana Bonomo, Bart Beverly, Fred Ramsdell and Kurt Brorson left the laboratory.

Honors, Awards, and Scientific Recognition

Dr. Schwartz is a member of the editorial boards of Science, International Immunology, Immunology Today and the International Journal of Cell Cloning.

During the past year he was a guest lecturer in the advanced immunology course at the Uniformed Services Medical School, an invited symposium speaker at the annual meeting of the French Society for Immunology, an invited speaker in the Immunology Seminar Series at the University of Washington in Seattle, WA, an invited speaker in the Biology Seminar Series at the California Institute of Technology in Pasadena, CA, an invited speaker in the Red Cross Hospital seminar series in Gaithersburg, MD, a guest editor for Volume 12 of the Annual Review of Immunology, an invited symposium speaker at the International Conference on Cellular and Molecular Aspects of Self Reactivity and Autoimmune Diseases held in Taormina, Italy, a lecturer in the American Association of Immunologists advanced course in Immunology in Colorado Spring, CO, the plenary lecturer at the Western Conference in Immunology in Berkeley, CA, and an invited speaker at the 14th International Congress of the Transplantation Society in Paris, France.

Dr. B. J. Fowlkes was awarded the National Institute of Health's Merit Award and was selected to the Editorial Board for the Journal of Experimental Medicine. She served on the Committee on the Status of Women of the American Association of Immunology, was appointed by the NIH Director to the Committee on the Status of NIH Intramural Women Scientists, and was appointed to serve as the Women Scientists' Advisor to the Scientific Director of NIAID. During the past year, Dr. Fowlkes was invited to chair two workshops on T Cell Differentiation, Subset Markers and Function held at the 8th International Congress of Immunology in Budapest, Hungary, to speak in the NIAID Research Grand Rounds at the NIH and gave plenary lectures on thymic selection at the Annual Meeting of the American Society of Transplant Physicians in Chicago, IL, and at the Symposium on T Cell Repertoires held in Arad, Israel.

Dr. Fowlkes was invited to give research talks at the University of Texas Health Sciences Center in San Antonio, TX; Dana-Farber Cancer Institute at Harvard Medical School in Boston, MA; University of Pennsylvania School of Medicine in Philadelphia, PA; University of California, Berkeley, CA; Johns Hopkins University School of Medicine, Baltimore, MD; and to lecture in immunology courses at the Uniform Services University of the Health Sciences in Bethesda, MD; the George Washington University School of Medicine, Washington, DC; and at Johns Hopkins University School of Medicine, Baltimore, MD.

Dr. Matzinger is a member of the editorial boards of Immunobiology, Seminars in Immunology, and the International Archives of Allergy and Immunology. During the past year Dr. Matzinger was invited to chair Workshop #53 at the 8th International Congress of Immunology in Budapest, Hungary and the minisymposium at the 1992 FASEB Conference in Anaheim, CA. She was also appointed to serve on a Ph.D. Thesis Committee at the University Hospsital in Leiden, The Netherlands.

Dr. Matzinger was an invited speaker at the European Research Conference "Self-Nonself Discrimination" in Como, Italy; Westinghouse Science Talent Search Finalists for the National Academy of Sciences, Washington, DC; European Conference "Therapeutic Immunodulation", Bad Windsheim, Germany; First Central European Immunology Summer School, Piestany, Czechoslovakia; Workshop on Retroviruses as Antigens and Superantigens in Autoimmunity and Tolerance, Basel, Switzerland; and an invited guest lecturer and workshop leader at the Public Affairs/Development/Administrators Forum of NCI's Comprehensive Cancer Centers and Clinical Cancer Centers, Columbus, OH. She was also invited to give research talks at the University of Pennsylvania, Philadelphia, PA, The Wistar Institute, Philadelphia, PA; University of Heidelberg, Germany; Basel Institute, Basel, Switzerland; University Hospital, Leiden, The Netherlands; Blood Bank, Leiden, The Netherlands; and at the NIAID Adopt-a-School Parternship Program, Dunbar High School, Washington, DC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00485-06 LCMI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald H. Schwartz	Chief	LCMI, NIAID
Others:	Scott Umlauf	IRTA	LCMI, NIAID
	Kurt Brorson	IRTA	LCMI, NIAID
	Satoru Kitagawa	Visiting Fellow	LCMI, NIAID

COOPERATING UNITS *(if any)*

Peter Linsley Bristol-Meyers Squibb

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	3.5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of T cell activation of normal murine T cell clones of the T_H1 type have shown that two signals are required to stimulate the cells to make interleukin-2 (IL-2) and divide. One signal is given through the antigen-specific receptor that is uniquely expressed on each clone. The other signal is called costimulation and is delivered through a different receptor. Our major objective during the past year was to characterize the costimulatory signal. Other laboratories had identified the B7/BB1 molecule as the inducible ligand on the APC and CD28 and CTLA-4 on the T cell as potential receptors for costimulation in the production of interleukin-2 (IL-2). We explored the role of these molecules in a new assay for costimulation, the induction of competence to respond to interleukin-4 (IL-4). Murine T_H1 clones do not respond directly to IL-4. They first must be activated by stimulating them with antigens and APC. After 24 hr. the activated T cells then proliferate in response to IL-4. We demonstrated that costimulation is required during the induction phase in order to make the clones competent to proliferate in response to IL-4. Although supplying costimulation also led to the production of IL-2, stimulation with this lymphokine was not the only signal required to gain competence, because addition of IL-2 to a T cell receptor occupancy signal only allowed the cells to become partially competent to respond to IL-4. We concluded from these experiments that the costimulatory signal contributes directly to the attainment of full competence to respond to IL-4. To explore the molecules involved in this costimulation, we used a recombinant fusion protein between CTLA4 and a human Ig immunoglobulin constant region. This molecule reacts with B7/BB1 expressed on activated antigen-presenting cells. We showed that CTLA4Ig could inhibit proliferation and IL-2 production of both T_H1 and T_H0 murine clones but not IL-4 production by the latter. In addition it inhibited the ability of T_H1 clones to gain competence to respond to IL-4. We concluded from these experiments that B7/BB1 is the critical ligand involved in costimulation for both IL-2 production and the gaining of competence to respond to IL-4.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00613-02 LCMI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

T Cell Subsets

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald H. Schwartz	Chief	LCMI, NIAID
Others:	Bart Beverly	Staff Fellow	LCMI, NIAID
	Albert Bendelac	Visiting Associate	LCMI, NIAID
	Delanie Cassell	IRTA	LCMI, NIAID

COOPERATING UNITS *(if any)*

David Alling LCI, NIAID

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	3.5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A universal problem in biology is the interpretation of the half-maximal response of a cell population to a stimulus. Either all the cells are producing only one-half of their maximal potential, or one half of the cells are producing at their full potential while the other half are producing nothing. To decide between these two alternatives we examined the production of interleukin 2 by single T cell clones at different concentrations of antigen. The response appeared to be quantal (all or more) but two different levels of response were observed, one approximately 3-4 times the other. Furthermore, the dose response curve was biphasic with a decrease in the number of cells in each group at higher (>30nM) antigen concentrations. Our interpretation of these results is that induction of transcription of the IL-2 gene is what is quantal. Thus, the group of cells giving the low level of IL-2 production had only one chromosome being transcribed, whereas the group giving the higher level of IL-2 production was transcribing both chromosomes. The fact that all the cells were not found in the high positive group at large antigen concentrations possibly reflects a negative feedback regulation on IL-2 transcription. Experiments are currently in progress to test these two hypotheses.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00486-06 LCMI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT

T Cell Differentiation

PRINCIPAL INVESTIGATOR	B. J. Fowlkes	Senior Investigator Section Chief	LCMI, NIAID
Others:	F. Ramsdell	NIH Staff Fellow	LCMI, NIAID
	E. Robey	Guest Researcher	LCMI, NIAID
	C. Pavlovich	Howard Hughes Med. Inst. Scholar	LCMI, NIAID
	E. O'Connell	Technician	LCMI, NIAID
	A. Nguyen	Summer Student	LCMI, NIAID

COOPERATING UNITS *(if any)*

Flow Cytometry Section, Biological Resources Branch, NIAID (F. Hausman); Animal Care Branch, NIAID (P. Golway).

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Thymocyte Differentiation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

During T cell development, thymocytes that are able to recognize self MHC are signalled to mature (positive selection), whereas those capable of self reactivity are selected to die (negative selection). Many of these studies deal with the paradox of how signaling resulting from a specific TCR-MHC interaction can determine the positive or negative selection events. Several investigations involving TCR transgenic mice reveal that distinct thymic antigen presenting cells are probably not responsible for these alternate forms of selection. Instead, they suggest that it is the affinity of the TCR-MHC interaction that controls the developmental outcome. Increasing the coreceptor expression (through a CD8 transgene) is able to enhance TCR recognition of MHC, and alter net selection effects from positive to negative. Likewise, enhancing MHC expression by increasing MHC gene dosage can alter developmental fate. Thymic selection can, in some cases, be determined also by tissue-specific peptides presented only on thymic epithelium.

Our previous studies indicated that the thymus is able to induce tolerance to self antigens not only by deletional mechanisms but also by clonal inactivation (anergy). Transfer of the nonresponsive cells into hosts that lack the self antigen or into in vitro culture results in a reversal of the nonresponsive state, indicating that antigen is required to maintain this nondeletional form of in vivo tolerance.

The mechanism responsible for commitment to the $\alpha\beta$ or $\gamma\delta$ lineage has also been approached using TCR transgenic mice. If TCR-β transcriptional regulatory elements are used to direct expression of the TCR$\alpha\beta$ transgenes, $\gamma\delta$ T cells do not appear. Instead, there appears a $CD4^-8^-$ cell bearing the TCR-$\alpha\beta$ transgenic receptor, but with the developmental, phenotypic, and functional properties of $\gamma\delta$ T cells. These results suggest that T cell functions may be associated with a specific lineage, irrespective of TCR usage. These findings have led to an investigation of the role of TCR transcriptional regulatory elements in lineage development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00581-03 LCMI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

T Cell Tolerance and Memory

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Polly C.E. Matzinger	Expert	LCMI, NIAID
Others:	Adriana Bonomo	Visiting Fellow	LCMI, NIAID
	Garrett Hisatake	Howard Hughes Scholar	LCMI, NIAID
	Albert Bendelac	Visiting Associate	LCMI, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Tolerance Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying various aspects of the birth, life and death of T cells. BIRTH-1) For years scientists have looked for a self-renewing stem cell in the thymus and failed to find one. We found that intact fetal thymuses, placed under the kidney capsules of SCID mice, continued to produce T cells for at least 6 months. We are now studying whether the loss of this stem cell activity seen in the older experiments is due to age of the cells, the age and/or irradiated state of the recipient thymus, or the *in vitro* purification treatments. 2) In the presence of IL-7, the differentiation of αβ, but not γδ T cells is reversibly blocked in thymic organ cultures. We hypothesize that the levels of this lymphokine may regulate the rate at which αβ T cells are produced.

LIFE:1) Using mice primed before or after thymectomy, we found that virgin mice can only be primed for about 5 months after thymectomy, but mice primed before thymectomy respond for at least 4 months, at which time they still contain large number of cells expressing a 'virgin' surface phenotype, among which are a large number of functionally immunized CTL. 2) CTL to some Ags (usually viruses) do not seem to need help from CD4 T cells while CTL to other ags, (usually cellular Ags), are strictly help dependent. We have postulated that CTL activation by dendritic cells or activated APCs may bypass CD4 help & that some viruses may directly activate APCs. To test this hypothesis, we have immunized animals with purified dendritic cells under conditions where CD4 T cell help is limiting. We found that this procedure works in some cases & not in others & are now characterizing the differences.

DEATH-1) We found that thymic epithelium (TE) induces tissue specific tolerance in developing thymocytes & that this tolerance does not extend to skin specific antigens. Yet an animal is tolerance of its own skin. We are ow determining whether this particular skin antigen is found on bone marrow cells. If not, then one must begin to ask how an individual is tolerant of its own skin. 2) By grafting 2 sets of congenic fetal thymuses into normal & SCID recipients, we found that the mature thymic T cell population may contain up to 10% recirculating T cells, that the recirculating population contains both activated & resting cells, with a rather high proportion of γδ cells. We are now testing whether the recirculating population ever exists the thymus again and whether, during its thymic sojourn, it is activated or tolerized by antigens not expressed in the thymus in which it matured.

LABORATORY OF HOST DEFENSES
1992 Annual Report
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PHS-NIH

Summary Statement

LABORATORY OF HOST DEFENSES

National Institute of Allergy and Infectious Diseases

October 1, 1991 to September 30, 1992

Introduction:

The focus of work in the Laboratory of Host Defenses is (1) to study the basic mechanisms of protection against bacterial and fungal infection; (2) to study the physiology, biochemistry and molecular biology of phagocytic cells (blood neutrophils, monocytes, eosinophils, and fixed tissue macrophages) in host defense against bacteria and fungi; (3) to determine the role and effect of cytokines on both phagocytic cells and upon the general process of inflammation; (4) to investigate the cellular, biochemical and genetic basis of diseases leading to recurrent bacterial and fungal infections, particularly diseases affecting phagocytic cells function (chronic granulomatous diseases, hyper-IgE-recurrent infection [Job's] syndrome, specific granule deficiency, leukocyte adhesion deficiency, cyclic neutropenia and others); (5) to develop new animal models of human diseases which lead to recurrent infections; and (6) to develop new treatments for host defense defects leading to recurrent infections including new antibiotics or cytokine therapy or prophylaxis against infection, new diagnostic approaches for infections, and the development of gene therapy for such inherited immune deficiencies.

During the past year the Laboratory has continued to expand upon clinical studies in patient cohorts collected at NIH over more than 20 years and performed studies in patients and normal volunteers regarding the physiology and biochemistry of phagocytic cells and cytokines in infection and inflammation. The oxidative microbicidal superoxide-generating NADPH oxidase, the microbicidal granule proteins, and the chemoattractant receptors of phagocytic cells were studied.

Clinical Studies:

A study completed in the previous year demonstrating the efficacy of prophylactic γ-interferon to reduce infection frequency in patients with chronic granulomatous diseases (CGD) entered phase IV this year following FDA approval of this treatment. This phase IV study is aimed at determining the long term safety for the patients and the continued effect of γ-interferon on both phagocytic cells and the inflammatory process. This treatment appears to be without adverse effects. A new double-blinded randomized trial was initiated to determine whether prophylactic oral itraconazole can reduce fungal infections in CGD. In related studies on the patient group with CGD, data was collated and published relating to infections with unusual fungal organisms, and also the urological, orthopedic, and surgical anaesthetic considerations related to CGD. We also demonstrated that use of steroids is effective in controlling the granuloma complications of CGD. We have also developed new molecular biology probes, functional assays, and antibodies which yield more rapid and accurate diagnosis of CGD and determination of genetic subtypes of this disease. In particular specific restriction length polymorphisms were identified in the p67phox CGD gene which can be used for genetic diagnosis.

In studies of patients with Mycobacterium avium intracellulare (MAI) a family group with likely X-linked susceptibility to MAI was identified, with possible lymphocyte defect. A protocol is in progress which is examining the use of γ-interferon to enhance host defense against MAI in this patient group. These studies may yield important information regarding the use of this cytokine in infections with MAI.

Studies of the use of γ-interferon in altering the humoral and cellular defects seen in HyperIgE-recurrent infection syndrome (HIE) are in progress. This and other studies of the use of γ-interferon in treatment of MAI and CGD noted above will yield important information about the use of this cytokine in various infection syndromes.

Clinical studies of normal volunteers and patients were initiated to determine the systemic and local changes in cytokines and phagocytic cells in response to both local inflammation modelled by experimental skin blisters and/or in response to bacterial endotoxin as a model of bacterial infections. The time course of several cytokines in blisters was documented. Patients with HIE had a ten-fold normal increase in tumor necrosis factor in local lesions yet showed normal systemic responses to endotoxin. High levels of tumor necrosis factor in pulmonary tissues of HIE patients during staphylococcal pneumonia might occur and cause the extensive tissue damage seen in this disease. In other studies administration of endotoxin to normal volunteers was associated with four-fold increases in circulating hematopoietic progenitor cells. This may reflect an important role for circulating progenitors in bacterial infections. Furthermore, endotoxin itself or the cytokines increased by endotoxin, such as IL6, may be useful agents to enhance the yield of progenitor cells harvested from patients for gene therapy, a major future direction for the laboratory.

Studies of a patient with neutrophil specific granule deficiency demonstrated that this patient has a defect in expression of all eosinophil granule proteins except eosinophil peroxidase, but demonstrated increases of this defective eosinophil population in response to administration of granulocyte-macrophage colony stimulating factor. This demonstrates that the lesion of specific granule deficiency extends to a defect of eosinophil granule protein production, but that actual production of these cells is unaffected. These studies demonstrate that there is a genetic regulatory element or elements common to both eosinophil and neutrophil granule development.

Pre-clinical studies of the development of new treatments:

Studies have been initiated to develop methods of gene transfer of normal genes for the phagocyte NADPH oxidase into hematopoietic progenitor cells of CGD patients defective in such genes. These studies are aimed at gene therapy for CGD. Defective retroviruses free of helper virus activity were developed which were capable of transferring expression of normal oxidase genes in CGD cells defective in such genes. Transfer was associated with new protein expression and in related studies up to 10% of cells (both Epstein-barr virus transformed B-lymphocyte lines from CGD patients as a one model and peripheral blood progenitor cell cultures as a second model) demonstrated correction of superoxide-generating capacity. This is an important first step in development of gene therapy for CGD and is a model system applicable to other genetic diseases of blood cells.

Studies are underway to develop a novel method of immunization for chlamydial infections involving injection of DNA. The methodology involves injection of DNA containing coding regions for antigenic proteins of chlamydia. The DNA is contained within vectors that have been constructed to allow insertion into cells at sites of injection and secretion or presentation by these cells of the antigens to induce a protective immune response. Vectors have been constructed and shown to transfect into mammalian cells. Studies will be done in mice. These studies may yield new methodology for immunization against infectious microorganisms.

Basic science studies of phagocytic cells:
Studies of the growth of peripheral blood progenitor cells in culture demonstrate that stem cell factor (c-kit ligand) greatly enhances proliferation of such cells, but acts only as a co-factor in the presence of other hematopoietic factors. In related studies it was demonstrated that in normal individuals the number of circulating blood progenitor cells is very similar suggesting that this is a highly regulated physiologic phenomenon. These observations are important for pre-clinical studies indicated above aimed at harvesting and targeting peripheral blood progenitors for development of gene therapy for CGD.

Recently, in our laboratory all of the protein components of the phagocyte superoxide-generating NADPH oxidase have been identified. The cytosolic factors include the previously identified p47phox and p67phox, while the third required cytosol factor was shown to be the low molecular weight GTPase, rac2. Rac2 associates with and may be regulated by a guanine nucleotide exchange inhibitor protein. In related studies the only membrane component of the oxidase was shown to be cytochrome b558. These studies also demonstrated that this protein is actually a flavocytochrome containing both a flavin and NADPH binding domain in its large subunit. In related studies deletion mutant forms of the p67phox protein were created demonstrating that the catalytic elements reside in the amino terminal 28 kilodaltons. These findings definitively demonstrate the minimum components of the oxidase, begin the process of defining functional domains in individual oxidase proteins and will facilitate both the study of cellular elements regulating the oxidase and the development of therapies to augment or inhibit oxidase function as needed.

Studies of the expression of eosinophil granule proteins as recombinant proteins in E. coli have allow the defining of sequence elements in these proteins which target the binding of a foldase (chaparone protein) groEL. These studies are likely to yield information about homologous mammalian chaparone proteins that may be involved in phagocytic cell granule protein synthesis.

Preliminary work has been accomplished toward the end of creating a murine transgenic model of CGD. The cDNAs and genomic DNAs encoding the murine homologues of the human NADPH oxidase proteins defective in CGD have been cloned. These clones will be used for homologous recombination to inactivate the murine genes in embryonic stem cells. This animal model will be useful in the study of inflammation, the role of oxidants in host defense and as a model system for the gene therapy project outlined above.

Studies of chemotactic receptors of human phagocytic cells has lead to the cloning of cDNAs encoding an Interleukin 8 receptor, two formyl peptide receptors, and an A2 adenosine receptor. In addition an IL8 pseudogene related to IL8 receptor A was identified and a formyl peptide receptor family of three genes was defined. The IL8 receptor family was localized to chromosome 2q34-q35 and the formyl peptide receptor family localized to 19q13.3. Analysis of these genes, and comparison with other chemotactic receptor genes indicates that they all belong to a sequence related family which is part of the superfamily of heterotrimeric G-protein coupled, 7 membrane spanning segment family which includes the adrenergic, muscarinic, and neuropeptide receptors. These observations and studies are important for understanding receptor mediated events in phagocytic cells essential to the rational development of therapy for treatment of inflammatory diseases.

Administrative, Organizational, and Other Changes:

No administrative changes in the Laboratory have occurred. Dr. John I. Gallin is the Chief and Dr. Harry L. Malech is the Assistant Chief of the Laboratory. There are no sections.

Honors, Awards and Scientific Recognition:

Dr. John I. Gallin received the U.S. Public Health Service award for development of the Orphan Drug interferon γ for the U.S. Public Health. Dr. Gallin served as President-Elect of the International Society for the Immunocompromised Host and is now President of this Society. Dr. Gallin continues to serve on the Editorial Boards of Cellular Immunology, Inflammation, Advances in Host Defense Mechanisms and Advances in Inflammatory Research and is a co-editor of the text, Inflammation with the 2nd edition to be published in 1992. He serves on numerous NIH advisory boards. In FY' 92 Dr. Gallin served as a Visiting Professor at numerous medical centers and was a Plenary Lecturer at numerous meetings.

Dr. Harry L. Malech is currently Section Editor of the Journal of Immunologic Methods. He also serves as the Chairman of the NIAID Clinical Research Protocol Subcommittee. Dr. Malech was appointed to Scientific Advisory Board of Cadus Pharmaceuticals. In Fy'92 he was a Visiting Professor at the University of Maryland School of Medicine, and was an invited main speaker at The 13th Edmond de Rothschild School in Molecular Biophysics on Chemotaxis of Cells and Unicellular Organisms program on the Chemotaxis of Leukocytes at The Weizmann Institute of Science, Tel Aviv, Israel.

Dr. Philip M. Murphy was elected a member of the American Society for Clinical Investigation, and received an NIAID Merit Award for outstanding accomplishments in the study of chemotactic receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-17 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies of Abnormal Host Defense

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
	Steven M. Holland, M.D.	Senior Staff Fellow	LHD/NIAID
	Ellen DeCarlo, R.N.	Research Nurse	LHD/NIAID

COOPERATING UNITS *(if any)*

Douglas Kuhns, PRI-Frederick Cancer Research Facility, K.J. Kwon-Chung, LCI/NIAID, Harvey I. Pass, Surgery Branch/NCI, Maria J. Merino, Laboratory of Pathology/NCI, McClellan M. Walther- Surgery Branch/NCI, Genentech, Inc.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7		

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of these studies was to study patients with abnormal host defense. In FY '92 studies focused on patients with abnormal phagocyte function. Particular emphasis was given to patients with chronic granulomatous diseases of childhood (CGD), who continue to be the subject of intense study. Other diseases that were studied include the Hyperimmunoglobulin-E recurrent infection (Job's) syndrome, neutrophil specific granule deficiency, leukocyte adhesion deficiency and other patients with recurrent infections who do not fall into a specifically defined disease category. Clinical investigations included: (a) the successful treatment of Exophiala dermatitidis infection with surgery, amphotericin B, flucytosine, and white blood cell transfusions, (b) a detailed evaluation of the urological manifestations of CGD with the demonstration that steroids are an important adjunct in the treatment of the urological manifestations of CGD (c) initiation of a phase 4 study evaluating long term effects of γ-interferon in CGD. We continue to monitor and expand these cohorts of patients who will serve as models for long term studies of the immunological manipulation of the abnormal host defenses and for studies developing gene therapy of immune deficiencies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00481-07 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Phagocyte NADPH Oxidase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Daniel Rotrosen, M.D.	Medical Officer	LHD/NIAID
Others:	Choh L. Yeung, B.S.	Biologist	LHD/NIAID
	Thomas L. Leto, Ph.D.	Senior Staff Fellow	LHD/NIAID
	Cheung H. Kwong, M.D., Ph.D.	Clinical Associate	LHD/NIAID
	Harry L. Malech, M.D.	Asst. Chief, LHD	LHD/NIAID

COOPERATING UNITS *(if any)*

Michael E. Kleinberg, M.D., Ph.D., Univ. of Maryland School of Medicine, Mark Levine, M.D., NIDDK

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to define the structure and biochemistry of the phagocyte NADPH oxidase, the electron transport chain responsible for production of microbicidal oxidants. This oxidase is comprised of membrane bound and cytosolic proteins which interact in the activated phagocyte to catalyze reduction of molecular oxygen to superoxide anion. Defects in any of several genes encoding oxidase factors cause chronic granulomatous disease (CGD) characterized by a failure to produce superoxide and recurrent life threatening infections. Knowledge of the structure of the oxidase and the biochemistry of oxidase activation are critical to longterm efforts aimed at gene therapy for CGD, development of novel agents to augment or blunt the inflammatory response, and to a molecular appreciation of signal transduction in diverse cell types. We have reconstituted oxidase activity in a cell-free system comprised of purified cytochrome *b* (an oligomer of gp91phox and p22phox subunits) and recombinant p47phox, p67phox, and GTP-bound forms of rac1 or rac2 (low molecular weight guanine nucleotide binding proteins) enabling detailed biochemical studies which were not previously possible. We have demonstrated that these protein components are necessary and sufficient for activation of the oxidase and have shown that cytochrome *b* contains flavin and heme redox centers. Cytochrome domains responsible for binding flavin and NADPH have been identified based on sequence homology to well characterized flavoproteins. Thus, cytochrome *b* is the sole electron transporting component of the oxidase and the cytosolic factors appear to function as regulatory elements that induce conformational changes in the cytochrome which enable it to transfer electrons. In the intact cell p47phox becomes heavily phosphorylated within a 10 kDa COOH-terminal region leading to interactions with a COOH-terminal domain of gp91phox (residues arg-gly-val-his-phe-ile-phe). Structural properties of side chains of arginine, valine, phenylalanine, and isoleucine within this sequence motif contribute significantly to protein interactions leading to assembly of the oxidase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00521-05 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Cytokines in Host Defense and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.,	Laboratory Chief	LHD/NIAID
Others:	Ellen DeCarlo, R.N.	Research Nurse	LHD/NIAID

COOPERATING UNITS *(if any)*

D Kuhns, Ph.D. PRI-Frederick Cancer Research Facility

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.5	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The kinetics of the appearance of cytokines and mediators of inflammation was determined in normal subjects and in selected patients with abnormal host defense. For this study a model of inflammation in the skin was employed using a blister device. In normal subjects C5a, LTB4, and γ-interferon appeared within 3 h of raising blisters, Il-8 and Il-6 appeared from 8 through 24 h and Il1-B, GM-CSF and TNF-α appeared from 12 to 24 h. In vitro studies indicated that exudate neutrophils are capable of TNF-α production. Studies in patients with chronic granulomatous disease revealed normal mediator accumulation in these patients. In contrast, patients with the hyperimmunoglobulin E-recurrent infection (HIE) syndrome had about ten-fold increased TNF-α accumulation in the blister fluid. The increased TNF-α was restricted to the local inflammatory response since in related studies, in which a preparation of the lipid A component of E. coli endotoxin was administered intravenously to normal volunteers and HIE subjects, normal increases in TNF-α, Il-8 and Il-6 in the circulation in HIE subjects was observed. Abnormal regulation of TNF-α at local inflammatory sites may have important implications in the pathogenesis of the post infectious complications seen in HIE such as bronchiectasis and bronchopleural fistula formation following pneumonia.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00614-02 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Phagocyte Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas L. Leto, Ph.D.	Senior Staff Fellow	LHD/NIAID
Others:	Cheung H. Kwong, M.D., Ph.D.	Clinical Associate	LHD/NIAID
	Isabelle Ossana de Mendez, Ph.D.	Visiting Fellow	LHD/NIAID
	Daniel Rotrosen, M.D.	Senior Staff Fellow	LHD/NIAID
	Michael C. Garrett, B.S.	Biologist	LHD/NIAID
	Anthony G. Adams, B.A.	Biologist	LHD/NIAID
	Harry L. Malech, M.D.	Assistant Chief, LHD	LHD/NIAID
	John I. Gallin, M.D.	Chief, LHD	LHD/NIAID

COOPERATING UNITS *(if any)*

P. S. Low, Department of Chemistry, Purdue University, West Lafayette, IN

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.4		

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is exploring structure-function relationships in important phagocytic cell proteins. Current efforts are focused on protein structural requirements for assembly of the NADPH oxidase, a system responsible for production of superoxide and its potent microbicidal metabolites. Using purified recombinant cytosolic oxidase factors p47-phox and p67-phox, biochemical complementation studies in the last year have defined all of the protein components necessary for in vitro oxidase reconstitution. These studies suggest that a membrane associated cytochrome b558 is the sole electron transfer component of the system, while three essential cytosolic proteins (p47-phox, p67-phox, and rac) are likely to serve in oxidase activation through signal transduction mechanisms common to all eukaryotic cells. We have purified two closely related rho subfamily members of GTP binding proteins (rac-2 and CDC42Hs) from neutrophil cytosol based on complemetation assays with recombinant p47 and p67-phox. We subsequently showed that recombinant CDC42Hs was not active and are now exploring the structural basis for the functional differences between these two G-proteins. Structural variants of all three cytosolic components are being produced and characterized with recombinant DNA expression systems. The roles of src homology domains (SH3) present in p47-phox and p67-phox are also being explored through genetic deletion and reconstitution studies. Using engineered forms p67-phox from a baculovirus expression system we have identified a minimal functional domain within the amino-terminal 244 residues of p67-phox and have shown that the SH3 domains are not essential for in vitro reconstitution. Related studies using mammalian vectors are addressing the roles of SH3 domains within intact cells. In separate studies, src homology domains were fused to tag sequences to identify the targets of SH3 domains in peptide and expression libraries and to study their role in subcelluar protein translocation.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00615-02 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Neutrophil Activation by Chemoattractants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip M. Murphy, M. D.	Senior Staff Fellow	LHD/NIAID
Others:	Jiliang Gao, Ph. D.	Visiting Associate	LHD/NIAID
	Sunil Ahuja, M. D.	Visiting Associate	LHD/NIAID
	H. Lee Tiffany, M. S.	Biologist	LHD/NIAID

COOPERATING UNITS *(if any)*

Uta Francke, M. D., HHMI, Stanford University School of Medicine
Elmer Becker, M. D., Ph. D., Dept. of Pathology, Univ. of Conn. Health Center

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the precise mechanisms by which neutrophils become activated by extracellular stimuli. The project focuses on a class of extracellular stimuli known as soluble chemoattractants. These include N-formyl peptides, C5a, and a large class of structurally related peptides known as intercrines. The intercrine superfamily includes the intercrine α family (interleukin-8, Groα, NAP-2, etc.) and the intercrine β family (MIP-1, RANTES, MCAF, etc.). We have previously established the 1° structure and signaling properties of the human N-formyl peptide receptor (FPR), FPRL1 (FPRL= formyl peptide receptor like) and interleukin-8 receptor A (IL-8RA) from cDNA cloning. We have now cloned the following genes: FPR, FPRL1 and FPRL2; IL-8RA, IL-8RB and IL-8RAP. Members of the FPR family have 58-81% aa sequence identity but differ markedly in binding affinity for N-formyl peptides; FPR/FPRL1 chimeras indicate that this is due to differences in the NH2-terminal half of the receptors. The 3 genes colocalize on chromosome 19q13.3 and lack introns in the coding block. Members of the IL-8 receptor family have ~80% aa sequence identity, also lack introns in the coding block and colocalize to chromosome 2q34-q35. IL-8RA is also a receptor for two other distinct intercrine α ligands, Groα and NAP-2; IL-8RAP is a pseudogene of IL-8RA; IL-8RB is a functional receptor for IL-8 and Groα. The recent extinction of IL-8RAP is an interestingly example of the evolution of the ligand-receptor relationship; at least five intercrine α ligands encoded by genes linked on 4q13-q21 have only two functional receptors encoded by genes on 2q34-q35. Finally, we have cloned the human A2 adenosine receptor cDNA and gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00644-01

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of Peripheral Blood Progenitors: a Target for Gene Transfer.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
Others:	Sudhir Sekhsaria, M.D.	Clinical Associate	LHD/NIAID
	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies peripheral blood progenitors (PBP) as a target for retrovirus mediated gene therapy of inherited diseases affecting the function of human phagocytic cells, including neutrophils and monocytes. PBPs which may include totipotent stem cells are similar or identical to the blood precursors in bone marrow, but circulate in small numbers in peripheral blood. PBP harvested and concentrated from peripheral blood by centrifugation apheresis have been used successfully by a number of investigators as an autologous transplant in lieu of or combined with progenitors harvested from bone marrow to reconstitute the bone marrow of patients with cancer treated with cytotoxic chemotherapy. Our hypothesis is that PBP harvested from patients with inherited immune diseases such as chronic granulomatous disease or leukocyte adhesion deficiency are a readily available target for functional correction by gene transfer. Using either whole peripheral blood or apheresis derived leukocytes enriched for PBP, we used monoclonal antibodies to enrich PBP several thousand fold yielding a population of cells where 40-90% of cells expressed the surface antigen CD34 characteristic of early blood progenitors. Using both liquid and soft agarose cultures we determined optimum conditions for growth and differentiation of PBP into colonies of mature neutrophils demonstrating a cloning efficiency of up to 10%. We showed that maximum proliferation occurred when the following growth factors were added to the culture: multilineage growth factor (MGF) (also called stem cell factor [SCF]), FGF-β, IL6, IL3, GM-CSF, and G-CSF. SCF acted as a co-factor to increase both the number and size of colonies. We also showed that the number of colony forming PBP in circulating blood was remarkably constant between individuals at 12 colony forming PBP/ml blood. This number increased from 2 to 4 fold in volunteers administered 3 ng/kg endotoxin. In related studies using the retroviral construct LXSN to transfer neomycin resistance to PBP we showed that under ideal growth conditions up to 30% of colony forming PBP cells could be transduced to acquire neomycin resistance. Thus, we have established methodology to harvest and purify PBP, to grow PBP in culture, and to transfer and express new genes in PBP. These represent important steps in the development of techniques for gene therapy of immune diseases affecting blood phagocytic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00645-01

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Therapy for Immune Deficiencies.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
CoPI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:	Sudhir Sekhsaria, M.D.	Clinical Associate	LHD/NIAID
	Julie Katkin, M.D.	Special Volunteer	LHD/NIAID
	Susan Dorman	Guest Reseacher	LHD/NIAID

COOPERATING UNITS *(if any)*

R. Michael Blaese, NCI; Richard Mulligan, Ph.D., Whitehead Institute.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies gene therapy for inherited immune diseases affecting human phagocytic cells. Our immediate goal is development of gene therapy for chronic granulomatous diseases (CGD). CGD are a group of 4 distinct genetic disorders with a common phenotype characterized by life-threatening recurrent infections and granuloma formation caused by failure of blood neutrophils and monocytes to produce superoxide, hydrogen peroxide and derivative microbicidal oxidants. Our initial studies have focussed on a type of CGD in which there is a failure to synthesize the p47^{phox} cytoplasmic component of the NADPH oxidase required for superoxide generation. We have developed two *in vitro* model systems to demonstrate the feasibility of genetic correction of p47^{phox}-deficient CGD. The first system involves the use of Epstein-Barr virus transformed B-lymphocyte cell lines (EBV-BL). EBV-BL from normal individuals produce small amounts of superoxide when stimulated, while those derived from p47^{phox}-deficient CGD patients do not produce superoxide and are missing p47^{phox} protein. Using the retroviral vector Lp47^{phox}SN we demonstrated that we could genetically correct the ability of the CGD EBV-BL to produce small amounts of p47^{phox}, but superoxide generation was not restored. Using a new vector MFG-p47^{phox} packaged into transducable virus envelope using the packaging line ψ-crip, we were able use this retrovirus to correct the CGD EBV-BL population to 7% of normal superoxide production with a single round of transduction and up to 15% of normal with several rounds of transduction. This trait was stable for many weeks of subsequent culture. In the second model system marrow-like myeloid progenitor cells harvested from the peripheral blood could be induced to proliferate and differentiate *in vitro* to mature neutrophils capable of superoxide production. Similar cells from p47^{phox}-deficient CGD patients could not produce superoxide, but MFG-p47^{phox} retrovirus transduction of these precursors from CGD patients resulted in correction of superoxide production in 7% of resultant neutrophils. We demonstrate *in vitro* that retroviral transduction can result in functional correction of one genetic form of CGD. Theoretically, the peripheral blood progenitor model system of gene transfer could be scaled up to a procedure capable of being used for *in vivo* gene therapy of this form of CGD.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00646-01 LHD

PERIOD COVERED

October, 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transgenic Animal Models of Human Immune Defects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven M. Holland, M.D.	Senior Staff Fellow	LHD/NIAID
Others:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
	Harry L. Malech, M.D.	Asst. Chief	LHD/NIAID
	Sharon H. Jackson, M.D.	Clinical Associate	LHD/NIAID
	Angela M. Toms, B.A.	Medical Technologist	LHD/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at understanding the role of specific enzyme systems in generating and maintaining host immunity. The approach taken involves the creation of mice with discrete defects in host defenses by using homologous recombination of DNA into the host genome to disrupt relevant genes. Lesions will be introduced that either correspond to previously identified mutations in humans or that incapacitate systems probably involved in host defense.

We have used the NADPH oxidase system as a paradigm for creation of mice with a genetic defect similar to one found in humans, chronic granulomatous disease (CGD). To this end, we have cloned, sequenced, and functionally expressed the cDNA of the murine homolog of the human 47^{phox} gene. We have isolated genomic fragments from this gene and are characterizing them for disruption and re-insertion into the mouse genome. This will provide the basis for a murine model of the most common form of autosomal recessive CGD. We have also cloned and characterized genomic fragments of the murine homolog of the human 91^{phox} gene. This will provide us with a tool for creating a murine model of the predominant, X-linked form of CGD.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00647-01 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genes and Gene Products as Immuno-Adjuvants.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven M. Holland, M.D.	Senior Staff Fellow	LHD/NIAID
Others:	John I. Gallin, M.D.	Lab Chief	LHD/NIAID
	Sharon H. Jackson, M.D.	Clinical Associate	LHD/NIAID
	Angela M. Toms, B.A.	Medical Technologist	LHD/NIAID

COOPERATING UNITS *(if any)*

Tom C. Quinn, M.D., LIR, NIAID

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1	PROFESSIONAL: 1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at understanding, characterizing, and evaluating a novel form of gene product delivery, direct DNA injection. Cytokines will be administered intramuscularly to mice in the form of either a cDNA expression vector or preformed protein in order to elicit a strong immune response. Simultaneously or in sequence will be administered expressible cDNAs for specific antigens from either *Chlamydia trachomatis* or *Chlamydia pneumoniae*. Animals will then be followed by evaluation of cytokine response in the circulation, local response to the injected materials at the site of administration, and development and type of immunity. To these ends we have obtained and/or engineered into expressible forms the cDNAs for murine TNF-alpha, murine IL-2, murine IL-4, and murine IL-6. These constructs have been shown to work in other systems and have been productively transfected in our hands. In order to develop a source for the chlamydial antigens needed for these studies, we have generated DNA libraries in a bacterial plasmid system from both *Chlamydia trachomatis* and *Chlamydia pneumoniae*.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00648-01 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NADPH Oxidase Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas L. Leto, Ph.D.	Senior Staff Fellow	LHD/NIAID
Others:	Richard T. Kenney, M.D.	Clinical Associate	LHD/NIAID
	Isabelle Ossana de Mendez, Ph.D.	Visiting Fellow	LHD/NIAID
	Michael C. Garrett, B.S.	Biologist	LHD/NIAID
	Harry L. Malech, M.D.	Assistant Chief, LHD	LHD/NIAID
	John I. Gallin, M.D.	Chief, LHD	LHD/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.2	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to explore the organization of genes encoding components of the NADPH oxidase. This enzyme is responsible for the generation of potent microbicidal oxidants derived from the free radical superoxide anion. The importance of this system in host defense can be appreciated from patients with chronic granulomatous disease (CGD), who suffer from recurrent infections due to genetic lesions in any one of four genes encoding essential protein components of the oxidase. This program will aid in our understanding of the molecular genetic basis for this hereditary immune deficiency and may suggest certain strategies for correction of these gene defects. Current efforts are focused on the gene encoding the cytosolic oxidase factor p67-phox, which was recently mapped to chromosome 1q25. In the last year we have isolated and mapped cosmid genomic clones spanning 70 kilobases within this gene locus. This gene is organized into 16 exon segments and corresponding intron boundaries have been located and sequenced. This work has provided insights on the molecular basis for p67-phox deficiency in one autosomal recessive CGD patient, where a deletion at an exon-intron junction predicts a splicing defect that would cause premature termination of protein coding sequence. Other work has defined the location and sequence of a normal restriction fragment length polymorphism (RFLP) within the p67-phox gene, which was used as a genetic marker in prenatal diagnosis of a healthy individual in a family with p67-phox deficient CGD. In addition to obtaining information regarding genetic defects causing CGD, other work has sought to define genetic signals governing myeloid specific gene regulation of p67-phox. A putative gamma-interferon consensus sequence binding protein (ICSBP) binding site was recognized in the genomic sequence upstream from cDNA base -68. Specific gene promoter activity will be studied using reporter gene fusion constructs which contain candidate regulatory sequences. This work may define mechanisms by which NADPH oxidase gene expression may be regulated by gamma-interferon and other immune modulators.

PPHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00649-01 LHD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Phagocyte Granule Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Helene F. Rosenberg, M.D.	Senior Staff Fellow	LHD/NIAID
Others:	Richard L. Griffith, M.D., Ph.D.	Clinical Associate	LHD/NIAID
	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the molecular events involved in the biosynthesis of neutrophil and eosinophil granules and granule proteins. The project focuses on the identification and characterization of the regulatory elements affecting the expression of granule protein genes and identification and characterization of the events resulting in granule protein folding and translocation and granule formation. Our recent work has shown that the defect resulting in the disorder known as neutrophil specific granule deficiency produces similar abnormalities in eosinophils, suggesting coordinate regulation of these two granulocyte lineages at this developmental stage. Further experiments designed to characterize the molecular defect underlying this disorder are described. In a separate set of experiments, we have described a novel interaction between two eosinophil granule proteins and the prokaryotic chaperone protein, groEL, and have localized a unique binding site near the amino terminus of one of the two granule proteins. Further experiments designed to isolate and characterize the homolog mediating the folding of these granule proteins in their native locale are described.

PHS 6040 (Rev. 5/92)

LABORATORY OF IMMUNOLOGY
1992 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1991 through September 30, 1992

Introduction

The goals of the Laboratory of Immunology (LI) are the elucidation of the fundamental mechanisms of the immune system; the application of the resultant knowledge to the understanding of the pathogenesis of immunologic disorders such as autoimmunity, immunodeficiency and allergic diseases; and the development of new approaches to immunization that would be useful in vaccine development. LI has made important progress towards these ends through the application of powerful new technologies that have revolutionized modern biomedical science. These include techniques of contemporary molecular biology, preparation and study of transgenic mice, development and use of monoclonal antibodies, cloning of normal and transformed lymphocytes and flow cytometric analysis and sorting. These methods combined with new techniques in protein chemistry and cell biology have allowed the solution of many of the major problems in immunology and should lead to important advances in efforts to prevent and treat diseases of the immune system and disorders caused by the action of the immune cells and antibodies.

Production of a Single-Chain Soluble Class I MHC Molecule that Expresses Biological Activity

Class I MHC molecules consist of two chains, a heavy chain encoded by the MHC class I gene, and a light chain, β2 microglobulin. Peptide binding occurs to a groove in the heavy chain, but the interaction appears to be stabilized by the binding of β2-microglobulin, presumably through control of the overall conformation of the molecule. LI scientists have now constructed a single chain molecule containing both the class I heavy chain and β2-microglobulin in a continuous polypeptide. The effort to design this molecule was guided by modeling the structure of the class I molecule. Such models indicated that likelihood the connecting the N-terminal residue of the MHC class I heavy chain to the C-terminal residue of β2-microglobulin using a 15 residue spacer consisting of $((Gly)_4Ser)_3$ would generate a molecule whose conformation mimicked that of the normal class I molecule. A polymerase chain reaction strategy was used to generate clones that coded for such single chain molecules. The resulting clones were transfected into expression systems and large amounts of the protein was obtained. Interestingly, the soluble class I molecule expressed cell surface epitopes associated with molecules in the native class I conformation and the purified single chain molecule proved capable of presenting antigen in an *in vitro* presentation system and could also stimulate an alloreactive T cell hybridoma (Ribaudo and Margulies, LI/NIAID; Mage, LB/ NCI).

Invariant Chain and Peptide Play Critical Roles in the Intracellular Transport and Membrane Expression of Class II MHC Molecules

In general, class I MHC molecules sample peptides present in the Golgi and endoplasmic reticulum while class II molecules predominantly sample peptides in endosomes. To understand how the "anatomic" specialization of class II molecules is achieved, LI scientists have studied the mechanisms regulating their intracellular trafficking and availability for peptide binding. An *in vitro* lysate system was developed through which the movement of class II-invariant chain complexes could be examined. These studies revealed that class II/invariant chain complexes have an unstable conformation but that they fail to bind peptide. When invariant chain is removed, the molecules form aggregates at low pH unless peptide-binding has occurred. If the class II molecules bind peptide, they adopt a stable conformation and can be transported to the cell surface.

These results thus suggest that class II MHC molecules undergo an "editing" process in an endosomal compartment as they lose invariant chain. Those that bind peptide are expressed on the cell surface in a stable form and become available for antigen-recognition by T cells whereas those that fail to bind peptide are targeted via aggregation for lysosomal degradation within the cell (Rinker and Germain, LI/NIAID).

Direct Binding of Soluble Purified Class I MHC Molecules to Antigen-Derived Peptides

The binding of antigen-derived peptides to class I MHC molecules is the critical event in the creation of the antigen complex recognized by CD8+ T cells. Although much progress has been made in the identification of peptides that bind to class I molecules, the actual binding process has been difficult to study because it involves cell-associated molecules. LI scientists have developed systems for the study of solution binding of purified, truncated class I MHC molecules to peptides in order to understand both the thermodynamics and kinetics of the reaction as well as to study its fine specificity and the contribution of β2-microglobulin to the binding process. Utilizing a spin column assay system with an iodine-labelled peptide, they have directly demonstrated the inhibitory effect of the addition of amino acids at the carboxyterminus of a self-peptide that binds to H-2L^d and have demonstrated the critical role of proline at position 2 and of leucine, phenylalanine or methionine at position 9 of the peptide. Recently, they have utilized surface plasmon resonance to directly measure the mass of protein that binds to an immobilized ligand on a dextran-derivatized gold film. This technique allows the measurement of binding of class I molecules to anti-class I antibodies immobilized on the film and, by using antibodies that discriminate between the peptide-bound and peptide-free form of class I, to indirectly measure peptide binding. Advances in the chemistry of coupling peptide to the gold film have now made possible the direct measurement of the binding of soluble class I molecules to immobilized peptide by surface plasmon resonance. The measurement of equilibrium and rate constants for the binding event and the direct determination of cross-reactivity of specific peptides for different class I molecules should now be straightforward. This approach should provide detailed understanding of the molecular mechanisms through which peptide-class I interactions occur (Boyd, Corr, Khilko, Lees and Margulies, LI/NIAID).

Serum Angiotensin-Converting Enzyme Processes Peptides, by Proteolysis, for Binding to Class I MHC Molecules

Class I MHC molecules bind peptides and the resultant complex is recognized by T cell receptors of CD8+ T cells. Studies of peptides recovered from class I molecules indicate that "ten-mers" are often the optimal-sized molecules for such interaction. In the study of the *in vitro* presentation of an HIV gp120 peptide from the IIIB isolate, it was observed that a 15-mer, p18, was capable of stimulating responses. However, such responses only occurred if fetal calf serum were included in the buffer in the antigen-presentation reaction. Even in the presence of an excess of exogenously added β2-microglobulin, p18 did not stimulate a response in the absence of serum. It was then shown that protease activity in the serum was hydolyzing the peptide and, in parallel, it was demonstrated that the optimal stimulatory peptide was a 10-mer. Detailed analysis of the proteolytic activity in serum that was capable of inducing the p18 to be a stimulant of T cell activation revealed that it could be inhibited by Captopril, known to be a specific inhibitor of angiotensin converting enzyme. These studies indicate that some peptide processing for antigen-presentation can occur extracellularly and that individuals who use inhibitors of angiotensin converting enzyme as anti-hypertensive agents may have diminished capacity to mediate such effects (Kozlowski, Corr, Boyd and Margulies, LI/NIAID).

Production of Soluble γδ T Cell Receptor Heterodimers, as Chimeras with Immunoglobulin Constant Regions

Little is known about the antigens that γδ T cell receptors bind to and whether such molecules are complexes with MHC-like proteins. Furthermore, virtually no information exists regarding the structure of these receptors. The availability of an active γδ receptor in soluble form would be a major step toward solving these enigmatic problems. To this end, LI scientists, with colleagues in the Biological Resources Branch, have succeeded in preparing chimeric genes that specify the VDJ complex and the extracellular domains of the γ or δ C region together with the hinge, C_{H2} and C_{H3} domains of human γ1 immunoglobulin H (IgH) chain. Upon transfection of COS cells with either construct alone, little product is secreted. However, transfection with both the γ and the δ constructs results in the secretion of a molecule of ~150 kD consisting of a heterodimer of the TCRγ-IgH and TCRδ-IgH chains. These chimeric proteins are immunoprecipitated both by antibodies that react with all γδ T cell receptors and by anti-clonotypic antibodies. Efforts are now underway to determine whether the secreted chimeric heterodimer expresses antigen-binding activity and to determine the nature of its ligand (Eilat and Shevach, LI/NIAID; Kikuchi and Coligan, BRB/NIAID).

Identification of CD4-Binding Regions of Class II MHC Molecules

The CD4 molecule plays a central role as a co-receptor on T cells through its interaction with class II MHC molecules. This interaction is important in the process of thymic selection, being critical to the development of mature T lymphocytes that are $CD4^+$,$CD8^-$ and also contributing to T cell activation in the periphery through the interaction of $CD4^+$ T cells and antigen-expressing antigen-presenting cells (APC). LI scientists have undertaken a study of the regions of the class II molecules that participate in the interaction with CD4 both to identify the critical molecular sites of this interaction and to compare it to the interaction of CD4 with gp120 of HIV. A large number of mutations were introduced into the genes for the α and β chains of the class II MHC molecule encoded by $I\text{-}A^d$. These mutations were generally centered in the α2 and β2 domains of the molecule. Mutations of a localized region of the β2 domain of the β chain were found to profoundly reduce the capacity of the class II molecule to functionally interact with CD4 for T cell activation. Molecular modeling reveals that this region in the class II molecule corresponds to a surface loop analogous to the region on class I molecules that interacts with CD8 (Koening and Germain, LI/NIAID).

Partial Agonists for the T Cell Receptor: Polymorphisms in Class II Molecules Lead to Partial Stimulants/Inhibitors of T Cell Clones

Responses of $CD4^+$ T cells are elicited as a result of the binding of a complex of peptide and class II MHC molecules to T cell receptors in association with a series of other interactions, collectively referred to as accessory function. It has generally been believed that the interactions of T cell receptor and its ligand that led to the occupancy of the same number of receptors would always result in the same outcome, as long as accessory stimuli were maintained constant. Somewhat surprisingly, it was observed that, in a system in which a T cell clone normally recognized cytochrome c in association with $I\text{-}E^k$, the introduction of as few as two residues from polymorphic sites of $I\text{-}E^s$ profoundly altered the response. In this system, the I-E expressing APC induced an alloresponse of the part of the T cell clone. When wild type $I\text{-}E^k$ antigen-presenting cells were used, the addition of cytochrome C peptide increased the response of the T cell clone as measured by enhanced IL-2 production. By contrast, when the mutant $I\text{-}E^{k/s}$ APC were used, the addition of cytochrome C peptide *diminished* T cell IL-2 production. However, the mutant APC supported increased IL-3 production and additional induction of IL-2 receptors on the T cell clone when cytochrome C peptide was added. It has been proposed that the complex of peptide and mutant class II molecules creates a partial agonist capable of transmitting only some of the stimulatory signals normally mediated by the interaction of antigen and the T cell receptor. One possible explanation is that the requirements for each type of response may vary and that the degree of stimulation mediated by the mutant class II molecule, possibly because of a more rapid dissociation rate, may be adequate to induce some but not other responses. The study of such partial agonists may allow a precise understanding of the control of activation in the T cell system and may suggest approaches for the development of therapeutic agents capable of regulating these responses in autoimmune disorders (Racioppi, van Meerwijk and Germain, LI/NIAID).

NF-κC, a Homodimer of the p50 Subunit of NF-κB, Represses the Function of the IL-2 Promoter

A 10 bp DNA sequence designated κB is a major regulatory element in the promoters of several immunologically important genes, including that for IL-2. LI scientists have developed a technique that allows the transfection of T cells from normal, non-transformed clones and thus permits the study of gene regulation in such cells. Using this technique, a novel nuclear binding factor complex was found, designated NF-κC. NF-κC interacts preferentially with a κB site in the IL-2 promoter. Indeed, the presence of NF-κC is inversely correlated with the expression of IL-2 suggesting it may be a negative regulator. Analysis of NF-κC reveals that it is a homodimer of the p50 subunit of NF-κB and that it directly represses the function of the IL-2 promoter. Furthermore, this complex also binds to a site in the HIV promoter, suggesting that NF-κC may repress both IL-2 and HIV transcription in resting lymphocytes. It has recently been shown that the binding activity of NF-κC is controlled by an inhibitory protein, I-κC, that sequesters the NF-κC complex. This molecular regulatory system for control of IL-2 and HIV transcription may have importance not only for the understanding of the physiologic regulation of IL-2 production but also in understanding the basis of viral transcription in HIV-infected T cells (Kang, Tran, Grilli and Lenardo, LI/NIAID).

Anergized T Cell Clones Show Diminished Induction of AP-1 Complex and Diminished Transcription of the IL-2 Gene

T cell clones stimulated with antigen/class II MHC complexes in the absence of accessory function enter a state of clonal anergy. These cells fail to produce IL-2 in response to stimulation with antigen and antigen-presenting cells although they are capable of responding to IL-2. Furthermore, they can produce some IFNγ and IL-3. The failure to produce IL-2 is associated with striking diminution in steady state levels of IL-2 mRNA, which appears to reflect diminished transcription of the IL-2 gene. LI scientists, and colleagues in LCMI/NIAID, have shown that of the recognized transactivator proteins of the IL-2 gene, only the AP-1 complex displayed diminished induction in anergized cells stimulated with antigen and APC. The importance of this finding in explaining the functional defect in anergized cells was verified by preparing stable transfectants of the T cell line expressing only the AP-1 enhancer and showing diminished activity of this construct in anergized cells. These results thus provide strong evidence that the molecular defect in anergy is the failure of T cell stimulation to cause production of the AP-1 complex and a resultant diminution in transcription of the IL-2 gene (Kang, Chen-Tran and Lenardo, LI/NIAID; Beverly, Brorson and Schwartz, LCMI/NIAID).

Interleukin-4 (IL-4) Controls the Differentiation of Naive CD4+ T Cells into IL-4-Producing Cells

$CD4^+$ T cells express much of their function through the production of a potent set of proteins designated lymphokines. In general, naive $CD4^+$ T cells produce interleukin-2 (IL-2) upon stimulation but little IL-4 or interferon-gamma (IFNγ). However, upon priming, $CD4^+$ T cells develop into cells that produce either IL-4 or IFNγ upon challenge, but generally not both.

Furthermore, this distinction in lymphokine-producing phenotype has major importance in determining the protective value of an immune response. In certain infections, the production of IFNγ is associated with a protective immune response while the production of IL-4 is associated with an ineffective response. LI scientists have made important progress in the understanding the factors that determine whether CD4$^+$ T cells will develop into IL-4 or IFNγ-producers. Using mice that are transgenic for T cell receptors specific for a cytochrome C peptide in association with I-E^k, they have shown that naive T cells respond to peptide with IL-2 production but, after in vitro priming, these cells can produce IL-4 or IFNγ. The principal factor determining which of these lymphokines they produce is IL-4 itself. If present in concentrations of $\sim 0.4 \times 10^{-10}$M or more during the priming process, the resultant cells produce large amounts of IL-4 and virtually no IL-2 or IFNγ. If completely absent, the primed cells make IL-2 and IFNγ and no IL-4. Intermediate concentrations of IL-4 usually lead to the appearance of both IL-4 and IFNγ-producing cells. Furthermore, under such conditions, the presence of IFNγ during the priming process will diminish the appearance of IL-4-producing cells and thus tilt the balance of the response toward the priming of IFNγ-producers. The critical role of IL-4 in this process has been demonstrated in *in vivo* studies in which treatment with anti-IL-4 at the time of immunization with a conventional antigen leads to striking diminution in priming for IL-4 production and often enhances priming for IFNγ-production. The effect of a single injection of anti-IL-4 at the time of immunization is observed for at least 100 days after priming and persists even after secondary challenge. These results indicate that the early effect of IL-4 in determining the character of an immune response is imprinted upon the system in a durable manner (Seder and Paul, LI/NIAID; Fazekas de St. Groth and Davis, Stanford University School of Medicine; Ben-Sasson and Gross, Hebrew University).

IL-4 Treatment of T Cells Suppresses IL-2 and IFNγ Production

The observation that IL-4 present during the priming process results in the appearance of cells that produce IL-4 but not IL-2 or IFNγ led LI scientists to examine the effect of treatment of T cells with IL-4 on early production of IL-2 and of IFNγ. They observed that the addition of IL-4 to T cells stimulated with concanavalin A or with soluble antibodies to CD3, a component of the T cell receptor complex, profoundly diminished the acute production of IL-2 and IFNγ. Analysis of mRNA levels indicate that the inhibition begins at about 12 hours after stimulation; the steady state levels of mRNA stimulated by anti-CD3 prior to that, although relatively low, are similar in cell populations stimulated in the presence or absence of IL-4. Thereafter, mRNA levels for IL-2 and IFNγ rise strikingly in cells stimulated in the absence of IL-4 whereas in cells stimulated in the presence of IL-4, mRNA levels for IL-2 and IFNγ actually diminish. Initial studies of half-life of IFNγ mRNA in stimulated cells indicate that it is in excess of two hours and not altered by treatment with IL-4 suggesting that the difference in production may relate to the failure of a late amplification in transcription to occur. The effect of IL-4 in inhibiting IL-2 and IFNγ production is a feature of both CD4$^+$ and CD8$^+$ T cells. An analysis of mechanism suggests that it is not mediated by interrupting accessory signals. Thus, in T cells stimulated with immobilized anti-CD3, anti-CD28 strikingly enhances IL-2 production and IL-4 does not block this effect of anti-CD28. Similarly, the inhibitory activities of CTLA-4-Ig, an inhibitor of the CD28/B7 accessory interaction, and of IL-4 appear to be independent further supporting the conclusion that IL-4 does not block accessory cell mediated stimulation. Current efforts are focussed on the possibility that IL-4 acts to alter the signal mediated by T cell receptor cross-linkage or that IL-4 interferes with the transcription of IL-2 (Hu-Li, Tanaka and Paul, LI/NIAID).

Interleukin-10 Mediates its Inhibition of Cytokine Production by its Action on Macrophages

Interleukin-10 (IL-10) was first identified based on its capacity to block production of interleukin-2 (IL-2) and interferon-gamma (IFNγ), but not interleukin-4 (IL-4), by cloned T cell lines. In order to examine the cellular basis of this inhibitory function, LI scientists have examined the effect of IL-10 on IL-2 production by resting T cells from normal donors stimulated with concanavalin A and varying types of accessory cells. It was found that both IL-2 production and T cell proliferation by CD4+ and CD8+ T cells was inhibited by IL-10 only if macrophages were used as accessory cells. When highly purified dendritic cells, activated B cells or L cells were used, IL-10 had no inhibitory activity. Moreover, the effect of IL-10 on inhibiting accessory cell function of macrophages did not appear to be due to the production of an inhibitory factor since dendritic cells caused excellent stimulation of T cells by concanavalin A in the presence of IL-10 and macrophages. These results suggest that *in vivo* the effects of IL-10 in regulating the pattern of lymphokine produced in immune responses may only be observed in the specific situations in which macrophages are the predominant antigen-presenting cells. This is most likely to occur in situations in which the T cell system is responding to obligate intracellular parasites infecting macrophages. Under these circumstances, IL-10 may play an important role in the complex process through which lymphokine-producing phenotype is established and maintained (Ding and Shevach, LI/NIAID).

IL-2 and IL-4 Treatment of Activated T Cells Sensitize Them for Apoptosis in Response to Antigen Receptor-Mediated Stimulation

LI scientists have shown that treatment of cloned T cells with interleukin-2 results in apoptotic death of the cells when they are subsequently challenged by antigen-receptor mediated stimulation. This effect also occurs with freshly isolated mouse T cells and occurs *in vivo* as shown by the protective effect of anti-IL-2 antibodies in superantigen-induced depletion of responsive T lymphocytes. It has now been demonstrated that IL-4 as well as IL-2 can sensitize T cell for apoptotic death and that the effectiveness of the sensitization step appears to depend on its capacity to drive the cell into particular phases of the cell cycle. Thus, aphidocolin, which arrests cells near the G1/S boundary, does not protect cells against lymphokine-induced receptor-mediated apoptosis whereas mimosine and desferoxamine, which block earlier in G1, exert a protective effect. These results shed further light on the basis of sensitization for apoptotic death and provide pharmacologic agents that may protect cells against the sensitization process (Boehme, Critchfield and Lenardo, LI/NIAID).

Infection with *Nippostrongylus brasiliensis* Renders T Cells from Anergic Mice Capable of Producing IL-4 but not IL-2 in Response to *In Vitro* Challenge

The role of infectious agents in overcoming anergic states and the relative capacity of such cells to produce IL-2 and IL-4 was examined in a model system in which unresponsiveness to the superantigen staphylococcal enterotoxin B (SEB) was induced. Treatment of adult mice with SEB results in a transient expansion of $V\beta 8^+$ cells followed by a fall in the numbers of these cells below the levels observed in untreated mice. $V\beta 8^+$ cells from these mice fail to produce IL-2 when challenged in vitro with SEB, in contrast to $V\beta 8^+$ cells from normal donors. These cells also failed to produce detectable amounts of IL-4 upon *in vitro* challenge. In order to determine whether an infection might alter this anergic state, as a model for the effect of infectious agents in the initiation of autoimmunity, such anergic mice were infected with the helminth *N. brasiliensis*. Cells of the anergic type ($V\beta 8^+$ cells) showed normal expansion in response to parasite infection but they remained incapable of producing IL-2 upon subsequent *in vitro* challenge with SEB. However, these cells show an ability to produce IL-4 in response to in vitro challenge with SEB that was similar to that of mice that had not been rendered anergic to SEB. These results indicate either that infections such as those with *N. brasiliensis* can "break" an anergic state, but only for the production of IL-4 or that $V\beta 8^+$ cells initially stimulated in the course of treatment with SEB differentiate into precursors of IL-2 and of IL-4 producers and only the precursors of the IL-2 producers become anergized. The precursors of the IL-4 producers, however, require treatment with *N. brasiliensis* in order to secrete IL-4 upon challenge. In either case, these results suggest that the production of IL-4 may not be inhibited in instances in which tolerance is achieved and/or maintained through a process of anergy. After stimulants capable of inducing differentiation to IL-4 production, such responses occur and may play a role in the autoimmune process (Rocken and Shevach, LI/NIAID).

Direct Measurement of Immunoglobulin Switch Rearrangement and Determination of Basis of Early Switching Events

Immunoglobulin class switching is the process through which an immune response produces antibodies of varying classes but with the same combining site. Analysis of myelomas and hybridomas secreting IgG, IgA or IgE revealed that the VDJ gene, normally found upstream of the switch region of μ (Sμ) and of the constraint region gene for μ (Cμ), has been translocated so that it is immediately upstream of the C region gene for the expressed isotype and the intervening DNA has been deleted. This deletion appears to be mediated by a recombination event involving the S regions resulting in the appearance of a chimeric S region upstream of the expressed C region. For example, in myeloma cells secreting IgG1, a chimeric Sμ/γ1 region is observed upstream of Cγ1 and the DNA downstream of the breakpoint in Sμ and upstream of the breakpoint in Sγ1 has been deleted. It has been suggested that this DNA deletional mechanism for switching may explain only the end stage situation, observed in myeloma and hybridoma cells, and that the initial production of "switched" isotypes may depend upon other mechanisms such as trans-splicing of independent transcripts or a complex process of specific cis-splicing of very long transcripts. In order to determine whether S region recombination and DNA deletion occurred in the early phase of the switching process, LI scientists, working with colleagues in CBER/FDA, have developed a technique to measure the frequency of chromosomes containing an Sμ/γ1 region. This technique, digestion/circularization-polymerase chain reaction (DC-PCR), is based on restriction enzyme digestion of DNA from stimulated B cells and the self-ligation of this DNA to form circles. Primers for sequences 5' of Sμ and 3' of Sγ1 are then used to amplify the "circle joint". An amplified product can only be obtained from circles that contain chimeric Sμ/γ1 regions. By precisely quantitating the product, the frequency of chromosomes that have undergone deletional switching can be accurately determined. This technique has been applied to an *in vitro* switching situation in which B cells are stimulated with lipopolysaccharide (LPS) and interleukin-4 (IL-4). Within 2.5 days, cells expressing membrane IgG1 can be observed at a low frequency rising to plateau levels of ~40% by 4 days. The frequency of chromosomes bearing chimeric Sμ/γ1 regions in these cells is sufficiently high to explain even the earliest switch events. Indeed, cell sorting was used to prepare pure populations of mIgG1+ B cells from cells cultured for 2.5 days with LPS and IL-4. The frequency of "switched" chromosomes in this population was sufficient for there to be at least one such chromosome per switched cell. These results lead to the conclusion that deletional switching is sufficient to explain early switching events. If other switching mechanisms are utilized, they may be involved only in specialized situations (Chu and Paul, LI/NIAID; Max, CBER/FDA).

Cytotoxic T Cells That Arise in β2-microglobulin "Knockout" Mice in Response to Tumor Injection Express CD8

The recent introduction of gene targeting methods for embryonal stem cells and the consequent ability to prepare mice with homozygous deficiency in a given gene ("knockout mice") makes possible a detailed examination of the consequences of the absence of a particular gene. One of the first genes of immunological interest to be deleted was that for β2-microglobulin. Since the assembly of and peptide-binding by class I MHC molecules depends upon β2-microglobulin, the observation that these mice lack detectable class I expression and showed few if any CD8+ T cells was not surprising. LI scientists have now shown that, if powerfully stimulated by antigen, these mice can develop cytotoxic T lymphocytes that express CD8. β2-microglobulin -/- mice received intraperitoneal injections of P815 tumor cells. The mice rejected the tumor cells and all survived. Peritoneal exudates from these mice contained cells that were specifically cytotoxic for the injected tumor cells and, surprisingly, the peritoneal exudates contained 20-50% CD8+ T cells. These CD8+ T cells are derived from the recipient and fail to express class I MHC molecules. These results could reflect a class I-independent mechanism through which CD8+ T cells may emerge, either in the thymus or through an extrathymic differentiation pathway. Alternatively, they may represent the expansion as a result of a powerful antigenic stimulus of a very small number of CD8+ T cells that may have been generated by the recognition of cell surface class I molecules lacking β2-microglobulin. These results open new approaches to examine differentiation of CD8+ T cells and strongly indicate that caution must be used in interpreting results with knockout mice since minor pathways of development or activation may become dominant in such situations and obscure the normal physiologic mechanism (Apasov and Sitkovsky, LI/NIAID).

Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play important roles in the U.S. and international scientific communities. They serve on editorial boards of many scholarly publications, on the advisory boards of major organizations, and act as organizers and major participants in international and national scientific meetings.

LI scientists have major editorial responsibilities for scientific journals and publications. Dr. Ethan Shevach completed a five year term as editor-in-chief of the *Journal of Immunology* in 1992. He is also a member of the editorial boards of *Cellular Immunology* and the *Journal of Immunological Methods* and is an editor of *Current Protocols in Immunology.* He is a member of the Council of Biology Editors.

Dr. William Paul is the editor of the *Annual Review of Immunology.* He is an advisory editor of the *Journal of Experimental Medicine*, an associate editor of *Cell*, a contributing editor of the *Proceedings of the National Academy of Sciences* and a member of the editorial boards of *Immunological Reviews*, *The Molecular Biology of the Cell*, *Cytokine* and *Current Opinion in Immunology.* He is also a transmitting editor for *International Immunology*, a corresponding editor for the *Proceedings of the Royal Society, Series B* and a member of the editorial advisory board of *Advances in the Regulation of Cell Growth.*

Dr. Ronald Germain completed a five year term as a deputy editor of the *Journal of Immunology* and was appointed as an advisory editor of the *Journal of Experimental Medicine.*

Dr. Rose Mage is a member of the editorial board of *Immunogenetics.* Dr. David Margulies in an Associate Editor of the *Journal of Immunology* and an editor of *Current Protocols in Immunology.* Dr. Michail Sitkovsky is an associate editor of the *Journal of Immunology* and is co-editing a book entitled *Cytotoxic Cells: Generation, Recognition, Effector Functions, Methods* for Birckhauser, New York. Dr. Michael Lenardo is an associate editor of *Molecular and Cellular Biology.* Dr. John Inman is a member of the editorial board of *Analytical Biochemistry* and is an advisory editor of *Molecular Immunology.*

Dr. Paul is a member of the Medical Advisory Board of the Howard Hughes Medical Institute; of the Board of Scientific Consultants of the Memorial-Sloan Kettering Cancer Center; of the Committee to Visit the Division of Medical Sciences of the Board of Overseers of Harvard College; of the Advisory Committee of the Pew Scholars Program in Biomedical Sciences; of the Fellowship Committee of the Cancer Research Institute; of the External Advisory Committee of the Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto; and of the Scientific Advisory Board of the Child Health Research Center of the Duke University School of Medicine. Dr. Paul was a member of the National Academy of Sciences, U.S.A., 1992 Nominating Committee and served as a member of a scientific review committee of the Scientific and Academic Advisory Committee of the Weizmann Institute of Science. He co-chaired the Task Force on the Intramural Research Program of the National Institutes of Health.

Dr. Paul was the co-chairman of the Program Committee of the 1992 Mid-Winter Conference of Immunologists in Asilomar, California and is a member of the Conference's board. He is the Secretary-General and Chairman of the Executive Committee of the Ninth International Congress of Immunology, to be held in San Francisco in 1995.

Dr. Paul received the Tovi Comet-Walerstein prize of the CAIR Institute of Bar-Ilan University in June of 1992. During the past year he presented a Yamamura Memorial Lecture at Osaka University and the Edward C. Franklin Lecture at the New York University School of

Medicine. He was an invited lecturer at the XIV International Congress of Allergology in Kyoto, Japan, at the International Congress on Cytokines: Basic Principles and Clinical Applications in Florence, Italy, at the Conference on Physiologic and Therapeutic Effects of Cytokines in Ein Gedi, Israel, at the Mid-Winter Conference of Immunologists in Asilomar, CA, at the Keystone Conference on Hematopoiesis in Tamarron, CO, at the International Union of Biochemists Symposium on Biochemistry of Diseases in Nagoya, Japan, at the International Conference on Cellular and Molecular Aspects of Self-Reactivity and Autoimme Diseases in Taormina, Italy, at the New York Academy of Sciences' Third International Conference on Drug Research in Immunologic and Infectious Diseases, at the International Coinference on Ribonucleoproteins and Other Autoantigens in Vienna, Austria and at the Eighth International Congress of Immunology in Budapest, Hungary. Dr. Paul chaired a symposium on Immunoglobulin Class Switching at the annual meeting of the American Association of Immunologists in Anaheim, CA.

Dr. Germain is the Director of the NIAID Grand Rounds Program, is a member of the Scientific Advisory Board of the Ruggiero Ceppellini Advanced School of Immunology, Naples, Italy and is chairman of the American Association of Immunologists Membership Committee. He was an invited lecturer at the Symposium on Antigen Processing and Presentation in Taos, NM, at the Biology of Parasitism Course at the Marine Biological laboratory, Woods Hole, MA, at the Stony Brook Symposium on the Molecular and Developmental Regulation of the Immune System, Stoney Brook, NY, at the Marion Merrell Dow Symposium on T Cell Recognition of MHC and Peptide, in Cleveland, OH, at the Fundacion Juan March Symposium on the Structure of the Major Histocompatibility Complex, Madrid, Spain and at the Annual meeting of the Laboratory of Tumor Cell Biology, Bethesda, MD. Dr. Germain was an invited panel member of the Workshop on Sexually Transmitted Diseases Human Challenge Studies in Rockville, MD and for the NIH Intramural Initiatives in Gene Therapy and on the Genome Project.

Dr. Shevach received the Distinguished Alumnus Award from the Boston University School of Medicine and a Distinguished Service Award from the American Association of Immunologists. He was honored by being named a Distinguished Lecturer of the National Science Council of the Republic of China (Taiwan). He is a member of the Scientific Advisory Board of the American Leprosy Foundation. Dr. Shevach was a member of the Organizing Committee, a Session Chairman and an Invited Lecturer at the Fourth International Conference on Lymphocyte Activation and Immune Regulation, in Newport Beach, CA and was an invited lecturer at the Conference on Intestinal Inflammation: Mechanisms and Modulation in Florence, Italy and at the Conference on T Cell Activation in Health and Diseases in Arad, Israel.

Dr. Mage is a member of the Board of Directors, Foundation for Advanced Education in the Sciences. Dr. Margulies is a member of the American Cancer Society, Immunology and Immunotherapy Advisory Committee, of the Arthritis Foundation, Fellowship Subcommittee, and of the Howard Hughes Medical Institute, National Institutes of Health Research Scholars Program Committee. He received a USPHS Outstanding Service Medal. Dr. Sitkovsky co-chaired a minisymposium on Cytotoxic Cells at the Annual meeting of the American Association of Immunologists in Anaheim, CA and was an invited speaker at the Eighth International Conference on Second Messengers in Glasgow, Scotland. Dr. Lenardo was an invited lecturer at the Gordon Conference on Immunochemistry and Immunobiology.

Administrative, Organizational and Other Changes

A Section on Biochemistry and Immunopharmacology was established in the Laboratory of Immunology with Dr. Michail Sitkovsky as its chief. Dr. Ronald Germain was promoted to the ES-4 level in the Senior Executive Service.

The Laboratory of Immunology continues to be a major training center for young immunologists. During the past year several scientists completed post-doctoral training or sabbatical periods in the Laboratory. Among these were David Agus, David Chin, Hsaio-Kun Chu, Richard Gala, Mariagrazi Grilli, Motohiro Hino, Yasuo Ishida, Kwang-Ho Lee, Luigi Racioppi, Kevan Roberts, Hirotaka Sugiyama, Toshio Tanaka, David Wilde, and Zheng-Sheng Ye. Each of these individuals made important contributions to the Laboratory of Immunology research program. It is anticipated that they will have very productive research careers and that many will attain leadership positions in modern immunology or related fields. Tragically, one of the most able and successful of the post-doctoral fellows in the Laboratory, Corine Layet, was killed in an automobile accident.

During the past year, several scientists joined the Laboratory of Immunology as post-doctoral fellows or sabbatical visitors. They include Stefan Boehme, Adriana Bonomo, Jeffrey Critchfield, Sergey Khilko, Nobuhira Oh-hori, Shigeru Ohno, Peter Rabinovich, Victor Rostopshov and Lin-Xin Zheng. It is expected that they will continue the tradition of excellence established by a long series of outstanding trainees in the Laboratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AI00030-24 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI, NIAID
Others:	Charles Chu	Staff Fellow	LI, NIAID
	Jeffrey Thomas	Guest Researcher	LI, NIAID

COOPERATING UNITS *(if any)*
CBER, FDA (E. Max); Roswell Park Memorial Institute (V. Chapman)

LAB/BRANCH
Laboratory of Immunology

SECTION
General Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Immunoglobulin class switching is the mechanism through which a given antibody combining site can be expressed on antibodies that mediate distinct biologic functions. An assay has been developed that allows the quantitative measurement of the degree of switch region recombination. This technique, digestion-circularization polymerase chain reaction (DC-PCR) was used to measure the frequency of class switching in vitro in cells stimulated with lipopolysaccharide and IL-4. It was shown that sufficient numbers of switched chromosomes existed to explain virtually all the Ig class switching implying that other methods such as transplicing or alternative splicing of full length transcripts were not required to explain the class switching even in the earliest phases of the process. The mapping of the gene for the murine x-linked immunodeficiency gene (*xid*), which controls B cell responses to polysaccharide antigens was begun. The approach used was high resolution mapping utilizing probes specific for polymorphisms to type the alternative forms of PCR products from an extended set of loci flanking xid. Once the gene is located precisely, "walking" through a *Yac*-library will be undertaken with the goal of identifying the *xid* gene itself and gaining functional information regarding the gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00035-17 LI

PERIOD COVERED

October 1, 1991 to Se[illegible]r 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Design and Synthesis of Immunomodulators, Immunogens, and Vaccine Constructs

PRINCIPAL INVESTIGATOR *(List other [illegible]nal personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John K. Inman	Section Chief	LI, NIAID
Others:	Andrew Lees	NIH Special Volunteer	Dept of Medicine, USUHS
	Patricia F. Highet	Technician	LI, NIAID

COOPERATING UNITS *(If any)*

Dept of Medicine, USUHS (F.D. Finkelman, J.J. Mond); New York Univ Sch Medicine (P.K.A. Mongini); Food & Drug Admin (B. Golding)

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.4 (1.0 not paid through NIH)	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed toward the design and synthesis of immunogens and immunomodulators for collaborative studies in basic and applied immunology. Much of this work has been focused upon (1) studying mechanisms of activation of B cells through their specific antigen-binding receptors (membrane immunoglobulins), (2) developing novel and highly effective ways to stimulate specific humoral and cellular immune responses, and (3) translating these findings into potentially practical approaches to the immunization of humans against infectious diseases (e.g., malaria, AIDS, diphtheria-pertussis-tetanus, and Haemophilus influenzae type B infections).

The basic studies on B-cell activation through antigen receptors have employed a large number of conjugates of anti-immunoglobulins (anti-receptor antibodies) that were prepared in this Section. Multiple antibody molecules have been covalently coupled to molecules of soluble, high molecular weight polymers to yield functionally multivalent conjugates that effectively cross-link antigen receptors on resting B cells. Soluble conjugates permit study of dose-response relationships and the quantitation of signal amplification due to multivalence by direct comparison with unconjugated, bivalent antibodies. Amplification of early activation events (from small resting B cells to DNA synthesis or S phase entry) through multivalent cross-linking of antigen receptors has led to 10-fold higher responses at less than 1000-fold lower doses of antibody. These conjugates have served as convenient models for T-cell-independent type 2 antigens which typically present multiple, identical antigenic epitopes to responding B cells. Collaborative studies have focused upon early activation and signal transduction events in *in vitro* experiments with enriched populations of normal, resting (murine and human) B cells.

New reagents and methods, developed in this Section, have been employed for coupling antigens and antibodies to one another or to functionalized polymers for investigating some novel approaches to synthetic vaccines. Enhanced humoral immune responses have been observed to heterodimers of antigen and anti-membrane proteins and to polyvalent conjugates of antigens with soluble, high molecular weight polymers (e.g., dextran). Rapid, large, specific antibody responses have been elicited with these constructs which were given to experimental animals by various routes without adjuvant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00036-27 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Visiting Scientist	LI, NIAID
	P.D. Weinstein	IRTA Fellow	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)* LOM, NIDR (N. Harindranath); Basel Inst Immunol, Basel, Switzerland (A. S. Kelus); Dept Microbiol /Immunol, Univ. Illinois Coll Med, Chicago (W.C. Hanly); DAD, USAMRID, Ft. Detrick, Frederick, MD (A. O. Anderson); Dept Biol Sci, Florida State U., Tallahassee, FL (K. Roux); Dept Tumor Cell Biol, St. Jude Children's Res. Hosp., Memphis, TN (L. M. Hendershot); Dept Microbiol, Med. Coll. Ohio, Toledo, OH (D. W. Metzger); Spring Valley Labs. Inc. (R. Shaw)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We use techniques of classical immunogenetics and of molecular biology to study the genetics of rabbit immunoglobulins (Igs), T cell receptors (Tcr), and related genes such as the recombinase activating genes RAG-1 and RAG-2 which are necessary for gene rearrangements to occur during lymphocyte development. We investigate the development of germinal centers and the regulated expression and sequence diversification of Ig genes during lymphoid cell development. Four VH-CH recombination sites map 3' of the VH genes and 5' of the JH genes. There appear to be two or more "hot-spots of recombination" within or near stretches of repetitive DNA in the DH-containing region of the rabbit. A fifth recombinant (R7K) has a site that maps 3' of the exons encoding the membrane terminus of the IgM heavy chain (Cμ) and 5' of Cγ. The exact site of this recombination and its possible localization in a region homologous to the region in other species containing exons for the IgD heavy chain is currently under investigation. In contrast to most mammals studied, membrane-IgD has not been definitively identified on rabbit B cells. We have identified two surface immunoglobulin complexes on rabbit peripheral blood B cells that possess heavy chains of similar apparent molecular size. These may represent complexes with rabbit IgM and IgD. Four proteins are found noncovalently associated with the mIg in each of the receptors. These include 42, 37 and 36 kD proteins that may be the rabbit homologues of murine Ig-β (B29) and Ig-γ and Ig-α (mb-1). These proteins are found associated as 75 kd and 115 kD heteromeric complexes. Two larger glycoproteins (100 and 150 kD) may be specifically associated with the non-μ receptor. The Ig-associated proteins vary in B-lymphocytes isolated from spleen, blood, bone marrow and gut associated lymphoid tissue (GALT). Lectins are allowing us to dissect rabbit GALT into subcompartments based on local accumulations of cells expressing particular oligosaccharides. Normal and VH-expression mutant *ali* rabbits show similar staining patterns;the defect in *ali* rabbits which leads to the expression of a different set of VH genes does not appear to affect the general architecture of GALT.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AI00134-30 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Richard Asofsky	Section Chief	LI, NIAID
Others:	J. Little	Staff Fellow	LI, NIAID
	F. Zhao	Visiting Associate	LI, NIAID
	H.-K. Chu	Visiting Associate	LI, NIAID
	N. Ohori	Visiting Fellow	LI, NIAID
	A. Brooks	Biological Lab Tech	LI, NIAID

COOPERATING UNITS *(if any)*
M. Flack, DEB, NICHD; H. Coon, LG, DCBD, NCI

LAB/BRANCH
Laboratory of Immunology

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TH2.2 is a cloned hybrid cell line (B lymphoma x B lymphocyte) which can be induced by bacterial lipopolysaccharide (LPS) to a reduced rate of growth, partial terminal differentiation accompanied by secretion of at least 4 products-IgM, GM-CSF, IL-3, and IL-6. When cells are cloned in the presence of LPS, individual clones are obtained in a wide range of sizes and with many secretory phenotypes. Clones can be obtained that make no products or 1, 2, 3, or 4 products in any combination. None of these phenotypes breeds true. Clones of different size have similar rates of growth. When analyzed by limiting dilution each clone over a 30 fold range of size gives rise to subclones in a wide range of sizes with a similar median in each case. Analysis of clones which are restricted in respect to secreted product show no clone which maintains restriction - most revert to the parental (TH2.2) type following passage in normal growth medium. Most clones are fully sensitive to the inducing effects of LPS when tested shortly after selection and expansion in growth medium. A few clones are found which are resistant to LPS in respect to inhibition of growth, but in most cases this resistance is lost gradually on continued passage in growth medium. One partly resistant line gives rise to resistant and sensitive subclones in growth medium, and these in turn give rise to both sensitive and resistant clones. LPS induces a state of resistance to itself which can be maintained if LPS is added to the growth medium, but which is lost gradually in the absence of LPS. These studies demonstrate an extensive epigenetic regulation of response phenotype in individual cells, and a cellular "memory" of LPS which is partly maintained with cell division.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AI 00224-11 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptors, Co-Receptors, and Counter-Receptors for T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	L. Ding	Visiting Fellow	LI, NIAID
	M. Rocken	Visiting Associate	LI, NIAID
	G. Perreira	Guest Worker	LI, NIAID
	R. Gala	IPA	LI, NIAID

COOPERATING UNITS *(if any)*
Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil

LAB/BRANCH
Laboratory of Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The interaction of the T cell receptor (TCR) with its specific ligand, peptide-MHC, is only one component of the events required for the activation of antigen-specific T cells. The primary goals of our studies are to characterize cell surface antigens (co-receptors) and their soluble or cell associated ligands (counter-receptors) which play critical roles in cell-cell interaction and in the interaction of T cells with their environment. We have concentrated our efforts in a number of distinct areas: 1) We have previously demonstrated that the human CD59 antigen and its murine homologue, Ly-6, play important roles in the T cell activation cascade. We have engineered soluble forms of both CD59 and Ly-6 and demonstrated that these fusion proteins interact with both soluble and membrane bound IgM. These studies raise the possibility that Ly-6/CD59 may play critical roles in T-B cell interaction. 2) We have shown that the newly characterized cytokine, IL10, inhibits T cell activation by acting on macrophages and not other types of accessory (AC) cells. These studies suggest that IL10 inhibits the costimulatory function of AC by preventing the induction of a critical membrane molecule needed for macrophage-T cell interaction. Studies are in progress to identify this target molecule. 3) One model of immunologic tolerance involves the induction of peripheral T cell anergy by the injection of adult mice with Staphylococcal enterotoxin B (SEB). We have reversed the tolerant state in SEB-treated mice by a concomitant infection with the nematode, *Nippostrongylus brasiliensis* (Nb), which results in the generation of SEB-specific IL4 producing T cells. These results have important implications for the induction of autoimmune diseases following infectious diseases. 4) In order to extend our studies to the responses of human T lymphocytes to infectious agents, we have utilized T and B lymphocyte deficient SCID mice and reconstituted them with leukocytes from patients with the tuberculoid form of leprosy. This model is being developed to analyze the requirements for the induction of a primary immune response to *Mycobacterium leprae* by human cells in the SCID environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00226-11 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	Others: M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Visiting Scientist	LI, NIAID
	P.D. Weinstein	IRTA Fellow	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LOM, NIDR (N. Harindranath); DAD, USAMRID,Ft. Detrick, Frederick, MD(A.O. Anderson); Dept. Biol. Sci. Florida State U., Tallahassee, FL.(K.Roux); Spring Valley Labs.Inc. (R.Shaw).

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We study genes of the rabbit immune system by techniques of molecular biology and immunology. *Alicia* rabbits have a mutation in the immunoglobulin heavy chain locus that affects the expression of heavy chain variable region genes (VH) encoding the a2 allotype. Earlier, we found a relatively small deletion of a segment containing 3' VH genes with important regulatory functions, the loss of which leads to the *ali* phenotype. The 3' end of the VH locus probably plays a key role in regulation of VH gene expression in rabbits because VH gene(s) in this region are the targets of preferential VDJ rearrangements. Alternative mechanisms such as gene conversion may contribute to the diversification of the antibody repertoire in this species. We used the PCR technique to amplify the expressed VH genes of mutant *Alicia* and normal a2 rabbits of different ages, in order to determine the sequences of VHa genes coding for a2 molecules as well as VHa- genes coding for the a- molecules whose expression is elevated in mutant *Alicia* rabbit. We found that the products of the first functional VH gene (VH4a2) or VH4a2-like gene(s) were expressed in two to eight-week-old mutant *Alicia*; expression of both the VHx and VHy types of a-negative genes was also elevated but the relative proportions of VHx and VHy, especially VHx, decreased with age; meanwhile, the relative levels of expression of the VH4a2 or VH4a2-like gene(s) increased. The appearance of sequences resembling that of the VH1a2 gene which is deleted in the mutants may be due to gene conversions that altered the sequence of the rearranged VH4a2; rearrangement of upstream VH1a2-like genes may also occur later in development. We used reverse transcription and PCR amplification to isolate the complete coding region of the rabbit RAG-2 gene. Two cDNA clones from thymic mRNA were sequenced and compared to the sequence of a genomic DNA clone from Dr. K Roux. Genetic polymorphism of the rabbit RAG-2 gene was observed by sequencing and Southern anal-yses. Northern analysis of thymic mRNA showed that the rabbit RAG-2 transcript was almost twice the size of the homologous mouse transcript. Southern analyses using a probe from 5' of the RAG-2 coding region indicate that the rabbit has several exons contributing to the mRNA sequence. The rabbit mRNA for RAG-1 is of comparable size to those of mouse and man. A fragment of RAG-1 coding sequence has also been cloned by similar PCR techniques and the sequence compared with genomic RAG-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00349-09 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	R. Koenig	Visiting Associate	LI, NIAID
	S. Sadegh-Nasseri	Senior Staff Fellow	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LCMI, NIAID (R. Schwartz); Univ. of Chicago (A. Sant); Basel Institute for Immunol. (F. Ronchese)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	2.3	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Class II MHC (Ia) gene products play critical roles in a variety of T and B lymphocyte responses. Biochemical and functional analyses have been used to investigate the relationship between class II structure and the critical function of peptide antigen binding. The use of direct peptide binding techniques, together with transfectants expressing mutant class II molecules, has permitted the definition of a local peptide binding subregion in the murine I-E molecule, and the role of specific allelically polymorphic residues in controlling the quantitative and qualitative binding of peptide to this molecule. Other experiments with highly purified class II proteins have shown that class II undergoes a characteristic structural change upon binding of appropriate peptides. This change appears related to the ability of the class II proteins to act like fully folded globular proteins inside the cell. The kinetics of class II peptide binding have been re-examined and two distinct phases of binding identified; a rapid initial binding with a rapid off rate, and a slow accumulation of long-lived binary complexes. This accumulation is markedly augmented at the pH of the class II (endosomal) processing compartment.

In addition to binding peptide and being recognized by the clonally distributed T cell receptors, class II molecules participate in T cell selection in the thymus and activation in the periphery by interacting with the CD4 molecule that is also the receptor for HIV-1. We have used site-directed mutagenesis to define the site(s) of interaction of class II molecules with CD4, and demonstrated that a short continuous region in the β2 domain of the class II molecules plays a predominant role in this interaction. Together with the studies on class II folding, transport, and control of peptide binding by the polymorphic domain, these experiments will provide new insight into the molecular mechanisms involved in antigen recognition by and activation of T lymphocytes.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00394-07 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Charles Hoes	Biologist	LI, NIAID
	Maripat Corr	Medical Staff Fellow	LI, NIAID
	Rosemarie Hunziker	Sr. Staff Fellow	LI, NIAID
	Steven Kozlowski	Medical Staff Fellow	LI, NIAID
	David Chin	Howard Hughes Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LI, NIAID (R. Germain); MB, NCI (T.Takeshita, J. Berzofsky); LB, NCI (M.Mage, L. Lee)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	115

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this laboratory has been the MHC class I molecule, classically considered a molecular heterodimer consisting of a 46 kd polymorphic heavy chain, exemplified by the murine H-2K, D, and L molecules (analogous to the human HLA-A, -B, and -C molecules), that is non-covalently assembled with the 12 kd monomorphic light chain, β2-microglobulin. Our objectives under this project title have been to evaluate: 1) the influence of β2-microglobulin and serum proteases on functional and biochemical binding of MHC class I molecules to antigenic peptides; 2) the expression and function of domain and sub-domain fragments of class I molecules generated by recombinant DNA methods, and expressed in tissue culture cells; 3) the function of soluble MHC class I molecules in a transgenic mouse model system; and 4) the production and function of single chain MHC class I molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00403-09 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	L. Racioppi	Visiting Associate	LI, NIAID
	J. van Meerwijk	Visiting fellow	LI, NIAID
	M. Gregory	Special volunteer	LI, NIAID
	A. Fox	Research technician	LI, NIAID

COOPERATING UNITS *(if any)*
LMI, BRMB, DCT, NCI (L. Matis); Basel Institute for Immunology (F. Ronchese)

LAB/BRANCH
Laboratory of Immunology

SECTION
Lymphocyte Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.3	.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antigen-specific T lymphocyte activation occurs through the clonally distributed T cell receptor. The available set of αβ T cell receptors is dictated by both positive and negative intrathymic selection events. The molecular basis of this selection, in particular, how bias for self-MHC presented peptides is achieved without corresponding deletions of these clones during establishment of self-tolerance, is unknown. Furthermore, the quantitative and qualitative relationships between receptor occupancy and signaling for differentiation, whether in the thymus or of mature T cells in the periphery, is also poorly understood. This project uses cellular and molecular tools to study the development of the T cell repertoire and the activation of T cells upon ligand engagement of αβ and non-clonal surface receptors.

IL-2 (T cell growth factor) production by CD4 T cells is known to be very dependent on co-stimulatory signals distinct from those arising from occupancy of the clonally distributed αβ T cell receptor. Investigation of a prototypic Th1-type T cell clone specific for cytochrome *c* and $E\alpha E\beta^k$ unexpectedly revealed that addition of the known peptide ligand recognized by this cell interfered with alloantigen stimulation of IL-2 production by the clone. Inhibition by added peptide was not due to competition for creation of the alloantigen itself, to over-stimulation of the clone, or to interference with the generation of second messengers inside the T cell sufficient for other effector responses such as IL-3 production. Instead, peptide complexes involving the mutant class II molecule appeared to interact with the T cell receptor, interfering with either the production of or intracellular action of the critical co-stimulatory signals needed for IL-2 gene activation. These observations provide a new view of the relationship between T cell receptor occupancy and the response to such occupancy in the context of the T cell-antigen presenting cell interaction. This may provide an important clue to the control of thymic selection, as well as the initiation and deviation of immune responses. These findings may provide a new approach to the control of autoimmune diseases characterized by oligoclonal T cell responses and a means of controlling the type of effector responses elicited by component vaccine formulations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AI00425-08 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Lymphocyte Physiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.M. Chused	Senior Investigator	LI, NIAID
Others:	Y.A. Ishida	Visiting Fellow	LI, NIAID
	Shigeru Ohno	Visiting Fellow	LI, NIAID
	E.M. Brown	Biologist	LI, NIAID
	P.E. Hart	Biologist	

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunology

SECTION
Transmembrane Signalling Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive "feed forward" and "feed back" regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00427-08 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	S. Apasov	Special Volunteer	LI, NIAID
	M. Piper/Hunter	Research Technician	LI, NIAID
	Pearl Chen	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of our studies is to identify the key cellular populations which are involved in the anti-viral and/or anti-tumor cell-mediated cytotoxicity *in vivo* and to develop new experimental strategies to target these subpopulations of lymphocytes for immunomodulation. This will eventually lead to the new treatments of autoiummune diseases and/or cancer.

A) Cytotoxic CD8+T -lymphocytes (CTL) were considered to be an important part of anti-viral and anti-tumor immunity due to their ability to recognize and to kill specific target cells.However,very low percentage or no $CD8^+$ CTL were found in recently developed MHC class I-deficient(b_2m-/-) mice with no significant effect on the development or survival of such animals. This raised doubts about the functional significance of $CD8^+$ CTL.If CD8+ CTL were proven to be irrelevant in vivo,then the conceptual framework of immunomodulation had to be reconsidered.

To clarify the role of CD8+CTL in *in vivo* tumor rejection we asked if the b_2m-/- mice are able to reject intraperitonealy injected tumor cells (as was shown to be the case with normal mice) and what is the cellular composition of peritoneal exudates leukocytes (PEL) from such mice.

It is found that b_2m-/- mice do reject i.p. injected MHC class I bearing tumor cells. Surprisingly, analysis of PEL CTL obtained from i.p. tumor injected b_2m-/- mice revealed the presence of a large proportion of functional, tumor destroying $CD8^+$, $CD4^-$, $\alpha\beta TCR^+$, $CD3^+$, Thy-1^+, MHC class-I-negative CTL with strong MHC class I directed cytotoxic activity.

These results reconfirm the anti-tumor role of CD8+CTL, call for the careful studies of local accumulation of $CD8^+$ CTL in b_2m-/- mouse model and suggest the redundancy of MHC class I expression in normal animals.

B) During our studies of the role of extracellular ATP in CTL-mediated cytotoxicity we established that it is $MgATP^{-2}$ and not ATP^{4-}, that may be a "messenger" in CTL-target cell interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00493-06 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

B Cell Stimulatory Factor-1 (BSF-1)/ Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI, NIAID
Others:	Robert A. Seder	Medical Staff Fellow	LI, NIAID
	Achsah Keegan	Guest Researcher	LI, NIAID
	Jean-Louis Boulay	Visiting Fellow	LI, NIAID
	S. Z. Ben-Sasson	Visiting Scientist	LI, NIAID
	Michael Lenardo	Senior Staff Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

Stanford University (B. Fazekas de St. Groth and M. Davis); National Cancer Institute (J. Pierce); Asthma and Allergy Branch, NIAID (M. Plaut); Beth Israel Hospital, Boston (S. Galli and A. Dvorak); Allergic Diseases Section, NIAID (J-P. Kinet)

LAB/BRANCH

Laboratory of Immunology

SECTION

General Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.7	3.2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T cell response to a variety of immunogens and infectious agents are dominated by cells that produce either IL-4 or IFNγ. The means through which this preference is established has been studied in in vitro priming of T cells from mice transgenic for T cell receptor α and β chains encoding a receptor for cytochrome c peptide 88-104. The results obtained indicate that IL-4 itself is the major factor that determines the production of IL-4 and that IL-4 suppresses priming for IFNγ and IL-2 production and promptly suppresses production of these lymphokines. The inhibition appears to be due to a direct action of IL-4 upon activated but not resting T cells. IL-4 also plays a key role in vivo in determining lymphokine-producing phenotype. Treatment of mice with anti-IL-4 antibody at the time of priming with a conventional antigen strikingly suppresses production of IL-4 in response to in vitro challenge with that antigen for from 8 days to 105 days after initial immunization but does not diminish priming for IFNγ-production.

IL-4 is also produced by mast cells and other FcεRI$^+$ cells. It has been demonstrated that in spleen and bone marrow and in short term cultures of these cells in IL-3, the major IL-4 producing cells expresses FcεRI but lacks *c-kit*, indicating that the cell is not a mature mast cell and suggesting that it is a basophil or possibly a mast cell precursor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00520-05 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of T Lymphocyte Responses to HIV Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	A. Fox	Research technician	LI, NIAID

COOPERATING UNITS *(if any)*
MB, NCI (J. Berzofsky, T. Takeshita); Nippon Medical School (H. Takahashi)

LAB/BRANCH
Laboratory of Immunology

SECTION
Lymphocyte Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.4	.1	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To develop therapeutic and vaccine strategies to combat HIV-1, the cause of AIDS, it is essential to understand the cell-mediated immune response to this agent. To pursue this goal, murine model systems for immunization, isolation of antigen-specific T cells, and *in vitro* analysis of T cells specific for HIV-1 proteins have been devised. Structure-function studies on a class II-restricted determinant have revealed how a small subset of residues controls binding to MHC molecules, how additional residues regulate the epitopic structure necessary for T cell recognition, and how it may be possible to improve the potency of vaccine antigens by removal of interfering groups in the natural antigen. Restimulation *in vitro* of spleen cells from H-2^d mice immunized with recombinant vaccinia virus containing the HIV-1 (IIIB, MN, or RF) gp160 gene elicited CD8+ cytolytic T cells specific for amino acids 315-329. All responses were found in H-2^d mice, and the CTL elicited were H-2D^d restricted, but the CTL produced were highly specific and did not cross-react on target cells incubated with peptides corresponding to the sequences of the different isolates. To understand the relationship among these and other HIV isolates in terms of CTL specificity, variant peptides representing either natural isolates or synthetic combinations of residues from the natural sequences were prepared and used as immunogens or target antigens. These studies showed that the CTL responses to HIV fall into groups related by the chemical nature of key epitopic residues in the inducing peptide. Further, alternation in the peptide structure between immunization and boost broadened the CTL response and suggests a strategy for immunization in man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00545-04 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	P.Romagnoli	Visiting Associate	LI, NIAID
	E.Bonney	Medical Staff Fellow	LI, NIAID
	F. Castellino	Visiting Associate	LI, NIAID
	C. Layet	Visiting Associate	LI, NIAID
	A. Rinker	Research Technician	LI, NIAID
	L-Y. Huang	Research technician	LI, NIAID

COOPERATING UNITS *(if any)*
Molecular Biology Section, LI, NIAID (D. Margulies, R. Ribaudo, S. Kozlowski); LVD, NIAID (J. Yewdell); Univ. of Oslo (O. Bakke); Mt. Sinai School of Medicine (E. Bikoff); Columbia College of Physicians and Surgeons (E. Robertson) Lab Immunology

LAB/BRANCH
Laboratory of Immunology

SECTION
Lymphocyte Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	3.3	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

αβ T cells respond to complexes of peptide antigen and MHC molecules. The way in which protein antigens are transformed into peptides suitable for such binding and the intracellular pathways followed by MHC molecules both before and after peptide association are critical to our understanding of T cell immunity. We have used normal and gene transfected cells to examine the issues. Class II molecules are efficiently assembled and transported in cells deficient in MHC class I molecule folding and stability due to an absence of peptide transporters in the ER membrane, indicating no absolute requirement for ER peptides or invariant chain in promoting initial class II dimer assembly and ER egress. However, these cells and other transfected fibroblasts are inefficient in exporting MHC class II out of the ER unless invariant chain is present. Only the lumenal and transmembrane portions of invariant chain are needed for this chaperone function, whereas the cytoplasmic tail of invariant chain is critical for guiding class II molecules to the endosomal pathway. Invariant chain has the capacity to alter the structure of early endosomes and change the rate of movement of endocytosed proteins through this early sorting compartment. Deletion of 10 residues in the invariant chain cytoplasmic tail retains the endosomal targeting capacity while eliminating the ability to modify early endosomes. In this case, invariant chain and class II enter late endosomes, and the invariant chain that accumulates here, in contrast to that in early endosomes, is cleaved in the lumenal region. Because Langerhans cells show what seem to be related changes in class II-invariant chain localization and endosomal structure concordant with alterations in the efficiency of peptide loading, we suggest that invariant chain and class II movement through the endosomal pathway is an event regulated by progressive proteolytic deletion of signals in invariant chain. This regulation may have a role in the normal physiological control of the efficiency of the class II processing pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00565-03 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Family of kB Regulators for Genes in the Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	K. Bruhn	Guest Researcher	LI, NIAID
	A. Chen-Tran	Biologist	LI, NIAID
	J.J.-S. Chiu	HHMI Scholar - U. Va.	LI, NIAID
	S.-M. Kang	HHMI Scholar - Harvard	LI, NIAID
	J. Nguyen	Summer Student, Univ. Md.	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Regulation of genes for several lymphokines as well as other molecules involved in the immune response depend on a 10 bp DNA sequence termed, kB. This sequence binds a growing family of nuclear proteins, several of which are related to the rel oncogene, that are capable of tightly governing transcription of these genes. Importantly, the kB sequence is found in the human immunodeficiency virus (HIV). A cardinal feature of the kB sequence is that it permits transcription in a highly regulated fashion both temporally and in appropriate cell-types for specific genes. We are attempting to elucidate how this specific regulation occurs. We have found the IL-2 receptor alpha chain gene enhancer acts specifically in T cells through cooperation of NF-kB and another gene regulatory protein called SRF.

We have also found that the microheterogeneity in DNA sequence among kB sites has regulatory significance. By studying normal non-transformed T lymphocyte clones we discovered a novel nuclear complex, termed NF-kC (previously called NF-CYT1) that interacts preferentially with a kB site in the interleukin-2 gene. The presence of NF-kC in a number of different biological conditions is inversely correlated with IL-2 gene expression in T cells. This suggests it may be a negative regulator. We have shown that NF-kC consists of a homodimeric complex of the NF-kB p50 subunit and that it can directly repress the function of the IL-2 promoter. Very significantly, this factor binds to the enhancer region of HIV. We postulate it may have a role in suppressing HIV viral transcription in resting T cells. Importantly, we have found that the binding activity of the NF-kC complex is governed by an inhibitory protein (IkC) that sequesters the NF-kC complex in the nucleus following antigen stimulation. Efforts are underway to molecularly clone the IkC molecule.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00566-03 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Regulatory Events in Establishing Mature T Cell Tolerance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	S.-M. Kang	HHMI Scholar, Harvard	LI, NIAID
	A. Chen-Tran	Research Technician	LI, NIAID
	Di Jiang	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LCMI, NIAID, Drs. Ronald Schwartz, and Bart Beverly

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	0.75	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T cell tolerance has been found to occur in immature T cells in the thymus and also extrathymically in mature T cells. Our studies of mature T tolerance has revealed that they may undergo deletion or enter a functionally unresponsive state termed anergy. In anergy, a T-helper cell can be induced to "turn off" IL-2 production if it is stimulated through its T-cell receptor in the absence of costimulation. T cell anergy cannot be demonstrated in the transformed T lymphoma cells typically used for gene regulation studies so we have developed a system to study gene regulation in antigen-specific nontransformed T lymphocytes. We found that decreased production of IL-2 bioactivity is reflected in decreased steady-state mRNA and is due to decreased transcription of the IL-2 gene in anergic cells. An analysis of the transactivator proteins in the IL-2 promoter, showed that the NFAT, NF-kB, and octamer binding proteins are induced to similar levels in normal and anergized cells, but there was a specific defect in the induction of AP-1. AP-1 is a gene regulatory complex that is comprised of the protein products of the proto-oncogenes c-jun and c-fos. In order to assess the functional role of this decrease in AP-1 activity, we produced a number of stable transfectants of A.E7 cells with various reporter constructs. Results indicate that a 300 bp enhancer fragment of the IL-2 gene and constructs bearing only the AP-1 site have decreased activity in anergic cells. These findings suggest an intriguing mechanism by which the protein products of two proto-oncogenes may participate in a mechanism of T cell tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AI00622-01 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro Measurement of Binding of Self and Antigenic Peptides to MHC Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Maripat Corr	Medical Staff Fellow	LI, NIAID
	Sergei Khilko	Visiting Associate	LI, NIAID
	Marina Khilko	Special Volunteer	LI, NIAID

COOPERATING UNITS *(if any)*
LMB, NIDDK (E.A.Padlan)

LAB/BRANCH
Laboratory of Immunology

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this laboratory has been the MHC class I molecule, classically considered a molecular heterodimer consisting of a 46 kd polymorphic heavy chain, exemplified by the murine H-2K, D, and L molecules (analogous to the human HLA-A, -B, and -C molecules), that is non-covalently assembled with the 12 kd monomorphic light chain, β2-microglobulin. Our objectives in this project have been to examine the interaction of self and antigenic peptides to the MHC class I molecule using several different systems and to evaluate these interactions in three dimensional computer modeling studies based on crystallographic MHC structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z0100623-01 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro Folding and Assembly of MHC Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Randall Ribaudo	Guest Worker/Staff Fellow	LI, NIAID
	Amit Golding	Guest Worker	

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunology

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this laboratory has been the MHC class I molecule, classically considered a molecular heterodimer consisting of a 46 kd polymorphic heavy chain, exemplified by the murine H-2K, D, and L molecules (analogous to the human HLA-A, -B, and -C molecules), that is non-covalently assembled with the 12 kd monomorphic light chain, β2-microglobulin. Our objectives in this project have been to develop an in vitro system for examining early events in the folding and assembly of the MHC molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AI00624-01 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
New Chemical Tools for Immunology and Structural Biology Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John K. Inman	Section Chief	LI, NIAID
Others:	Patricia F. Highet	Technician	LI, NIAID

COOPERATING UNITS *(if any)*
DCBD, Lab Pathology, NCI (H.C. Krutzsch); NIDR, NIH (F.A. Robey)

LAB/BRANCH
Laboratory of Immunology

SECTION
Bioorganic Chemistry Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project covers the design, synthesis and use of new reagents and chemical constructs that will meet specific requirements in research projects of collaborating groups or individuals. Research goals fall within the fields of basic and applied immunology and structural biology.

For the preparation of conjugates and immunoadsorbents, heteroligation strategies have played a central role our work. In these strategies, two disparate structures are cross-linked without self-polymerization by placing uniquely interacting functions on each component through chemical derivatization. We have favored the use of sulfhydryl and sulfhydryl-selective groups as the paired functions, with cross-linking occurring through formation of stable thioether linkages.

New reagents have been developed for the placement of sulfhydryl-selective, haloacetyl (chloro-, bromo- and iodoacetyl) groups onto proteins, peptides and polymers. In particular, we have introduced a reagent ("BBAL") for placing a bromoacetyl moiety at any desired sequence position of a synthetic peptide, so that the resulting peptide may be directly coupled to sulfhydryl-bearing carriers at this designated position. Peptides that are synthesized with both bromoacetyl- and sulfhydryl-bearing (cysteinyl) residues may be self-polymerized to produce immunogens, or cyclized under different conditions to yield conformationally constrained epitopes for grafting onto immunogenic carriers. We have designed and used improved iodoacetylating agents and also reagents for placing protected/activated sulfhydryl groups on oxidized polysaccharide chains of antibodies and other glycoproteins. The terminal sulfhydryl group is spaced from the polysaccharide chain by intervening structures in order reduce steric hindrance to the approach of macromolecular reactants.

We have recently designed, synthesized and tested a selective alkylating agent for cysteine side chains in proteins that permits positive identification of Cys residues as a phenylthiohydantoin (PTH) analogue in the course of microsequencing of amino acids in peptide fragments (at $\leq$ 10 pmole level). The polarity of the new, stable PTH derivative, resulting from the Edman degradation through Cys positions, is such that its HPLC peak falls in an unused portion of the standard chromatogram. This finding solves the major remaining analytical problem in the chemical structure determination of peptides/proteins at the ultramicro level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z0AI00625-01 LI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Phosphorylation in Effector Functions of T-Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	T. Sugiyama	Visiting Associate	LI, NIAID
	F. Redegeld	Visiting Fellow	LI, NIAID
	P. Rabinovich	Visiting Scientist	LI, NIAID
	S. Apasov	Special Volunteer	LI, NIAID
	M. Piper/Hunter	Research Technician	LI, NIAID
	Pearl Chen	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of our studies is to understand the biochemical pathways and to identify key proteins (enzymes) that are involved in the activation and the effector phase of such important immune responses, like T-cell-mediated cytotoxicity and lymphokine secretion. It is expected,that such knowledge will enable us to selectively manipulate different functional responses of T-cells with specifically designed immunomodulators.

We attempt to reach this goal by taking advantage of the (A) extensive knowledge of the structure of the well known cAMP-Dependent Protein Kinases (PKA) and by (B) developing new approaches for the isolation of novel, functionally important protein kinases.

A) In experiments designed to directly evaluate the role of the PKA's catalytic (Cα) subunit in Cytotoxic T-Lymphocytes (CTL) effector functions, cells were pretreated with antisense oligomers complementary to mRNA for the Cα subunit of PKA. In another approach the role of PKA in interleukin-2 (IL-2) production was studied by transfection of T-cells with Cα-inhibiting regulatory subunit (RIα) of PKA with dominant negative mutation and by using antisense Cα mRNA oligodeoxynucleotides.

The obtained results suggest,that the free Cα subunit of PKA down-regulates TCR-triggered, protein-synthesis independent responses like cytotoxicity and exocytosis, but is required for the protein synthesis-dependent processes like interferon or IL-2 secretion by T-lymphocytes. Thus, these results point to PKA as an attractive target for the immunomodulation.

(B) Identification of Ectoprotein Kinases in Lymphocytes.

While pursuing our "extracellular ATP" hypothesis of the cell-mediated cytotoxicity, we demonstrated the presence and characterized highly active and extracellularly located protein kinases (ectokinases). Their isolation and cloning promises to provide a very novel set of potentially functionally important proteins.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AI00626-01 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inhibitors of Protein Kinases (or Phosphatases) as Immunomodulators

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	F. Redegeld	Visiting Fellow	LI, NIAID
	P. Rabinovich	Visiting Scientist	LI, NIAID
	M. Piper/Hunter	Research Technician	LI, NIAID
	Pearl Chen	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunology

SECTION
Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies of cellular populations and of the role of individual proteins in effector functions of T-lymphocytes have obvious immunopharmacological implications since they identify the cell type and key proteins that shoud be targeted for efficient immunomodulation.

A. Synthetic Peptides Inhibitors/Pseudosubstrates as Modulators of Cytotoxic Lymphocytes. We tested if it is possuble to influence functional responses of intact cells using synthetic peptides which mimick pseudosubstrate sequences or inhibitory domains of known protein kinases. We found that some basic amino acid-containing synthetic peptide substrates of protein kinases strongly inhibited several cellular responses of CTL. Peptides which contain basic amino acids, but do not have substrate specificity determinants for protein kinase, were not inhibitory. The requirement for the relatively high concentration of peptides needed to demonstrate their effects may reflect their partial degradation by peptidases. The presented data support the model in which specific features of amino acid composition allow for the peptide internalization and interference with cellular functions. Future refinements of this approach include design of protease-resistant peptides and targeting protein kinases (e.g. PKA) using natural inhibitory protein amino acid sequences.

B. Enhancement of Cytotoxic T Cells Functions by an Inhibitor of Serine/Threonine Phosphatase, Okadaic Acid. A specific and potent inhibitor of protein phosphatases 1 and 2A, okadaic acid (OA) at low concentrations enhanced, whereas at higher concentrations inhibited CTL responses as expected if protein phosphatases and protein dephosphorylation were indeed involved in the TCR-mediated signal transduction and effector responses of CTL. The biphasic character of the effects of OA on CTL-TC interactions suggests the existence of at least two functionally distinct phosphatases in CTL: an inhibitory phosphoprotein phosphatases that should be considered as a participant in the down-regulation of the cell-cell interactions between CTL and TC and yet another phosphatase that is needed for the TCR-mediated transmembrane signaling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AI00627-01 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulatory Events in T Cell Development in the Thymus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	K. H. Lee	Visiting Fellow	LI, NIAID
	J. Züñiga-Pflücker	Jane Coffin Childs Fellow	LI, NIAID
	H.Schwartz	Coop Student, UC-Berkeley	LI, NIAID
	Y.-N. Chang	Visiting Student, Cambridge Univ.	LI, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Immunology

SECTION
Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Increasing evidence suggests important events in T cell maturation in the thymus are governed by specific gene regulation. In the thymus, precursor cells undergo a complex set of developmental events and emerge as mature T lymphocytes capable of specific antigen recognition. We have found that gene expression varies at different stages of thymic development both in a variety of transformed cell lines that represent discrete stages of thymic development as well in fetal thymocytes harvested directly from mouse fetuses. We are focusing on regulation of the genes for IL-2 and IL-2 receptor alpha and beta chains together with the function of various transcriptional transactivators. To do so, we have developed a method to introduce exogenous DNA into thymocytes in intact thymic lobes. During cultivation of such lobes in vitro, normal developmental changes take place allowing us to study gene regulatory changes dynamically. We are also trying to identify gene products associated with the commitment to the T cell lineage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AI00628-01 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen-Induced Apoptosis (Propriocidal regulation) of Mature T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	S. Boehme	Postdoctoral Fellow, BRMP,NCI	LI, NIAID
	Jeffrey Critchfield	HHMI Scholar - UC-San Francisco	LI, NIAID
	A. Mermelstein	Summer Student - New York Univ.	LI, NIAID
	H. Tran	Summer Student - Georgetown	LI, NIAID
	S. Baker	Summer Student - Wm. and Mary	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunology

SECTION
Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have found that stimulation of the antigen receptor of mature T cells following exposure of the cells to mitogenic lymphokines such as IL-2 leads to the induction of programmed cell death or apoptosis (propriocidal regulation). This process can lead to the deletion of specific antigen-reactive T cells without affecting bystander cells. We are interested in two aspects of this effect: 1) the molecular events that take place, and 2) the possibility of using this mechanism to pharmacologically induce the elimination of T cells involved in disease processes. Our results indicate that antigen-induced apoptosis can only occur at specific stages of the cell cycle. Moreover, we can demonstrate that appropriate immunization will lead to the suppression of immune responsiveness, most likely because the specifically reactive T cells have been eliminated.

Several types of diseases that arise from the over-stimulation of the immune system including: allergies, autoimmune disorders such as multiple sclerosis and rheumatoid arthritis, and the rejection of tissue grafts. Activated T cells are the driving force behind the destructive immune response in these diseases. Current therapies involve drugs such as steroids and cyclosporin A that impair, in a general way, T lymphocyte function. Therefore, one serious side effect of these therapies is to increase susceptibility to infections. We have discovered that the cytokine IL-2 is able to cause the death of T cells that are specifically stimulated through their antigen receptor. This previously unknown property of IL-2 should allow the specific elimination of certain receptor-bearing T cells which could form the basis for new clinical applications of IL-2. It is conceivable that disease-causing T lymphocytes could be eliminated while sparing helpful T lymphocytes. Thus, the severe immunosuppression which is a side-effect of current therapies could be avoided. Conversly, because of the recent evidence that apoptosis may underly the loss of CD4+ T cells in AIDS, our in vitro model of T cell apoptosis may prove to be an important system to test for drugs that block apoptosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AI00630-01 LI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of γδ T Cells in Immunoregulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	D. Wilde	Medical Staff Fellow	LI NIAID
	F. Lynch	Visiting Fellow	LI, NIAID
	C. Brando	Visiting Associate	LI, NIAID
	D. Salomon	Guest Worker	LI, NIAID

COOPERATING UNITS *(if any)*
Biological Resources Banch, NIAID, NIH (G. Kikuchi, J. Coligan)

LAB/BRANCH
Laboratory of Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

γδ T lymphocytes represent a minor subpopulation of T cells which are found only in small numbers in peripheral lymphoid organs, but which represent the predominant T cells in many epithelial sites (skin, tongue, gut, vagina). Although the overall structure of the γδ T cell receptor (TCR) appears to resemble that of the αβ TCR, very little is known about the nature of the antigen and the presentation molecules (restriction elements) recognized by the γδ TCR. Major goals of our studies are to characterize the ligand for the γδ TCR and to define the role of this T cell subpopulation in normal immunoregulatory processes. We have designed chimeric genes encoding the VDJC regions of the γ- and δ- chains fused to the hinge, CH2 and CH3 domains of human IgG1. Transfection of COS cells with these constructs resulted in the secretion of a soluble γδ heterodimer which reacted with both anti-pan γδ monoclonal antibodies (mAbs) as well as anti-clonotypic mAbs. The availability of this soluble TCR in large amounts should allow us to study the interaction of the γδ TCR with its target antigen. In order to further functionally characterize γδ T lymphocytes, we have analyzed the requirements for activation of polyclonal populations of γδ T cells isolated from newborn thymocytes. Large numbers of γδ T cells can be generated by stimulating CD4-CD8- TCR αβ- thymocytes with combinations of cytokines (IL1+2, IL2+7, IL1+7), but not with any of the cytokines individually. Although γδ cells failed to proliferate when stimulated with plate-bound anti-pan γδ mAb, the addition of certain cytokines (IL1, IL2, and IL7) resulted in a vigorous proliferative responses. These studies demonstrate that cytokine production by γδ cells may be less efficient than that of TCR αβ+ T cells which can be directly triggered by plate-bound anti-TCR mAbs. Furthermore, it raises the possibility that γδ T cells may be passively recruited into sites of inflammation by cytokines produced by αβ T cells. We have injected large numbers of γδ T cells into SCID mice and observed that following injection of γδ cells which bear the Vγ3 TCR, CD4 and CD8 expression can be induced on precursor cells of SCID origin. These experiments suggest that γδ T cells may influence the development of the αβ T cell lineage particularly during T cell ontogeny.

LABORATORY OF INTRACELLULAR PARASITES
Rocky Mountain Laboratories
Hamilton, Montana
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Annual Report
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1991 to September 30, 1992

Research Highlights

Scientists in the Laboratory of Intracellular Parasites (LICP) study infectious diseases caused by the obligate intracellular bacterial pathogens *Chlamydia trachomatis* and *Rickettsia rickettsii.* The laboratory focuses on two general areas of study: (i) biology of host parasite interactions, and (ii) the hosts immune response to these obligate intracellular pathogens. Molecular biology and genetic approaches are being used to characterize parasite surface components in relation to pathogenicity and for the study of parasite developmental biology. Parasite surface components that function in attachment to eucaryotic host cells have been identified and are being used for the rationale design of experimental recombinant or sub-unit vaccines for the prevention of human diseases caused by these intracellular pathogens. This work has led to the design of a synthetic peptide immunogen that shows considerable promise as an experimental prototype vaccine against chlamydial sexually transmitted diseases. Animal models are being used to define the role of host immunity in mediating chlamydial disease pathology. Chlamydial antigens that evoke deleterious immune responses have been identified and immunologic mechanisms that mediate immunopathology are being studied. Scientists in LICP have identified chlamydial gene(s) that function in regulating the intracellular differentiation of chlamydial replicative and infective forms. A more precise understanding of the genetic and biologic mechanisms that regulate chlamydial intracellular growth offers potential for the development of new non-immunological based therapeutic strategies for the control of chlamydial diseases.

Immunobiology of Intracellular Parasites Section: Harlan Caldwell and Hua Su of the LICP Immunobiology Section have mapped antigenically common T-helper and neutralizing B-cell epitopes of the chlamydial major outer membrane protein (MOMP); the most promising candidate antigen for the development of a vaccine to prevent sexually transmitted diseases caused by *C. trachomatis*. A synthetic oligopeptide corresponding to these antigenically common T and B-cell epitopes was shown to be highly immunogenic in sub-human primates and was found to elicit the production of serum antibodies that neutralized the *in vitro* infectivity of those *C. trachomatis* serovars that are epidemiologically important as causative agents of chlamydial STDs. Because *C. trachomatis* are pathogens of human mucosal membranes an effective vaccine against chlamydial STDs must be capable of evoking a sustained secretory neutralizing antibody (sIgA) response to prevent chlamydiae from colonizing the epithelial cells of the urogenital tract. Towards this goal, Scott Manning has expressed these defined MOMP T and B cell epitopes as fusions with both the A and B subunits of *E. coli* heat labile toxin (LT), a known potent stimulator of mucosal immunity. Future work will focus on the characterization of the structural and functional properties of the LT:MOMP chimeric molecules and testing their immunogenicity and vaccine efficacy in a sub-human primate model.

Richard Morrison, Dan Rockey, and Ying Yuan, have continued investigations on the immunopathogenesis of chlamydial diseases. Richard Morrison and co-workers have continued their investigations involving the pathogenesis of chlamydial disease by characterizing the antigenic properties of the chlamydial 60 kDa heat shock protein (hsp60), and by describing the effect of interferon-γ (IFN-γ) on chlamydial growth and antigen expression. The hsp60 has previously been identified as an antigen that elicits immune responses that are thought to contribute to the pathogenesis of chlamydial disease. Ying Yuan (Univ. Montana), R. Inman (Univ. Toronto), S. Witkin (Cornell Univ.), R. Jones (Indiana Univ.) and Morrison have identified immunodominant regions of the hsp60 polypeptide by using amino terminal truncated hsp60 polypeptides in an ELISA. Using a panel of anti-hsp60 monoclonal antibodies (mAbs) three patterns of immunoreactivity were observed: chlamydial species specific, chlamydial genus specific, and cross reactive. The majority of the mAbs reacted to the amino or carboxyl terminus, and were found to be useful for the immunodetection of chlamydial inclusions, and immunoaffinity purification of recombinant hsp60. Sera from patients having different clinical manifestations of chlamydial disease were analyzed for anti-hsp60 antibodies. High titered anti-hsp60 antibody was observed in patients with severe chlamydial disease. The anti-hsp60 response was predominantly chlamydial-specific (i.e. no cross reactivity to the *E. coli* hsp60 homologue), and the immunodominant regions of the hsp60 polypeptide were the amino and carboxyl termini and amino acid residues 200 to 300. Dan Rockey and Morrison have prepared and tested purified recombinant MalE/hsp60 fusion polypeptides, that consist of portions of the chlamydial hsp60, for their ability to elicit systemic (dermal) and local (ocular) delayed hypersensitivity responses. Neither full length nor recombinant fragments elicited dermal DTH. Local lymphofollicular hyperplasia was elicited by full length hsp60, but thus far none of the recombinant fragments have been shown to elicit such a response. Persistent chlamydial infection could serve as the antigenic stimulus for the chronic inflammation associated with severe chlamydial disease. Morrison in collaboration with Gerald Byrne (Univ. Wisconsin) have shown that interferon-γ (IFN-γ) caused persistent chlamydial infection in vitro. Persistence was characterized by the development of noninfectious atypical intracellular forms that differentiated into infectious progeny when IFN-γ was removed. Furthermore, the atypical intracellular forms expressed near normal levels of hsp60, a deleterious immune target, and a paucity of the major outer membrane protein, a protective immune target. If IFN-γ affects chlamydial growth similarly in vivo, then persistently infected cells might function as depots of antigen for the stimulation of the chronic inflammatory responses that precede severe chlamydial disease.

Host-Parasite Interactions Unit: The Host-Parasite Interaction Unit has focused on the regulatory mechanisms governing the complex life cycle of *Chlamydia trachomatis* and on the structure and function of rickettsial surface components. Ted Hackstadt, unit leader, has developed an affinity purification procedure for an elementary body specific DNA binding protein from *C. trachomatis*, determined N-terminal amino acid sequence of that protein, termed Hc1, and cloned the encoding gene. Sequence analysis of the cloned gene revealed some remarkable properties for its expressed product including a high lysine content (29%), a correspondingly high pI, and significant homology to the H1 class of eukaryotic histones. Furthermore, a monoclonal antibody to this chlamydial histone-analog displayed immunoblot and antinuclear specificity suggestive of cross-reactivity to H1 histones. The gene is expressed only during the late stages of the chlamydial life cycle concomitant with the reorganization of chlamydial reticulate bodies into elementary bodies. Clifton Barry has expressed Hc1 in *E. coli* and demonstrated by both light and electron microscopy a compaction of the chromatin that ultrastructurally resembles late events in the chlamydial developmental cycle. Immunoelectron microscopy using anti-Hc1 antibodies localized Hc1 to the condensed nucleoids. Isolation of the nucleoid from *E. coli* expressing Hc1 verified association of Hc1 with the bacterial nucleoid and confirmed that Hc1 plays a major role in condensation of the chlamydial nucleoid that is characteristic of mature EBs. Hc1 expression in *E. coli* contributes to a global downshift in transcriptional activity which is also characteristic of mature EBs. Detailed biophysical analysis of the Hc1 proteins interaction with DNA indicate that it preferentially binds supercoiled DNA. The possible role of this DNA-protein interaction in more subtle regulatory events in the chlamydial developmental cycle are under investigation. Timothy Brickman has cloned, sequenced, and expressed the second *C. trachomatis* histone analog. This protein also displays primary sequence homology and antigenic cross-reactivity with eukaryotic H1 histones. It is likewise expressed only late in the developmental cycle consistent with its putative role in DNA structure. Purification procedures for this polypeptide are being developed.

Significant progress has been made in defining gene structure of surface proteins that are the target of neutralizing antibodies. Bob Gilmore has completed sequencing of the rOmp B surface protein to reveal that this protein, with an apparent molecular mass of 135 kDa, is encoded by an open reading frame sufficient to encode a protein of 168 kDa. The rOmp B protein seems to be highly conserved among spotted fever group rickettsia and is antigenically and structurally related to the more diverse typhus group rickettsiae. Ted Hackstadt, in conjunction with Witold Cieplak (LVP) has determined the N-terminal amino acid sequence analysis of a 32 kDa surface protein to establish that it is encoded by the 3'-end of the rOmp B gene. The rOmp B gene product therefore appears to be proteolytically processed to yield two apparently stable outer surface exposed components. The putative precursor has not yet been detected on virulent rickettsiae but an avirulent mutant apparently fails to cleave rOmp B since it lacks the 32 kDa peptide fragment and displays a rOmp B protein of the size predicted for an uncleaved precursor. The 135 kDa rOmp B and the 32 kDa fragment appear to remain stably, but non-covalently, associated on the rickettsial surface since immunoprecipitation by monoclonal antibodies specific for the 135 kDa portion of the molecule precipitates both fragments. The association of these polypeptides on the rickettsial surface may be involved in maintenance of a proposed paracrystalline surface array. Paul Policastro has identified the transcriptional start site for the rOmp A and rOmp B messages and constructed plasmids with the rickettsial promoters controlling chloramphenicol acetyl transferase reporter genes to demonstrate that rickettsial promoters are highly active in *E. coli*. Primer extension analysis of the RNAs has confirmed that the promoters recognized by *E. coli* are the same as those recognized by actively growing rickettsiae. These studies will provide a rational basis for attempts to express rickettsial genes in recombinant hosts. Gilmore has also examined the gene structure of the rOmp A protein by restriction endonuclease mapping and Southern blot hybridization. A portion of the gene expressing up to 13 direct repeat units is conserved among all but one species of spotted fever group rickettsia. Diversity in gene structure is demonstrable, however, by restriction endonuclease cleavage patterns or by polymerase chain reaction product size even between closely related members of single species. Both the expressed proteins appear to be highly toxic to recombinant hosts. Efforts are continuing to express these proteins in alternate hosts for evaluation of their functional roles. Sita Awasthi has described elements of signal transduction in the initial interaction of chlamydiae and rickettsiae with host cells. To date she has identified phospholipase C activity, phosphokinase C activity, inositol phosphates, phospholipase A2 activity, and metabolites of arachidonic acid metabolism such as prostaglandins in response to infection by intracellular parasites. Chlamydial internalization and growth is resistant to an extensive battery of tyrosine phosphokinase inhibitors. Preliminary evidence suggests that the signal transduction cascade accompanying interaction of intracellular parasites with host cells may initially involve G-protein activation. Bob Heinzen joined the laboratory in January and is investigating the interaction of rickettsiae with host cytoskeletal components. Through a combination of cytochemical techniques and electron microscopy, he has demonstrated an association of spotted fever group rickettsiae with actin. This actin association is specific to the spotted fever group of rickettsiae and not observed in the typhus group. These date indicate that cell-to-cell transmission of spotted fever group rickettsiae may involve actin polymerization similar to that described for *Shigella flexneri* and *Listeria monocytogenes.*

ADMINISTRATIVE REPORT

The LICP is divided into two sections: (i) the Host-Parasite Interactions Unit and (ii) the Immunobiology of Intracellular Parasites Section. The Host-Parasite Section is headed by Dr. Ted Hackstadt and the Immunobiology Section by Dr. Harlan Caldwell. Scientists working in the Host-Parasite Section include: Dr. Robert Gilmore (Staff Fellow), Dr. Paul Policastro (Senior Staff Fellow), Dr. Clifton Barry (IRTA), Dr. Tim Brickman (IRTA), Dr. Robert Heinzen (IRTA), and Dr. Sita Awasthi (Visiting Fellow). Scientists working in the Immunobiology Section include: Dr. Richard Morrison (Research Microbiologist), Dr. Hua Su (Visiting Associate), Dr. Scott Manning (Staff Fellow), Dr. Dan Rockey (IRTA), and Dr. Todd Cotter (IRTA). Drs. Heinzen and Cotter joined the laboratory this fiscal year. With the addition of Drs. Heinzen and Cotter the total FTE and non-FTE scientific slots allotted to LICP are occupied. Summer students working in LICP include: Kathy Olson, Janelle McLean, and Jennifer Rosquist. The following people were invited, by LICP staff, to present seminars: Dr. Robert B. Jones (Indiana University, Indianapolis, Indiana), Dr. Mike Minnick (University of Montana, Missoula, Montana), Natalie A. Marshall (Univ. of Texas Medical Branch, Galveston, Texas), Todd W. Cotter (Michigan State University), Gregory G. Burrows (University of Innsbruck, Austria), and Scott Boitano (University of California Los Angeles, Los Angeles, California). LICP organized and hosted a workshop on *In vitro* Neutralization of Chlamydiae, which was conducted at the RML on July 20-24. The workshop was sponsored by the Edna McConnell Clark Foundation and consisted of twenty scientists who represented chlamydial laboratories located both in the US and abroad. LICP also hosted the tenth sesqui-annual meeting of the American Society for Rickettsiology and Rickettsial Diseases. Over 80 scientists from the United States, Europe, and Asia attended this three day meeting. Scientific sessions covered epidemiology, ecology, cell biology, pathogenesis, genetics, and immunology of rickettsiae and related organisms.

HONORS AND AWARDS

NIH Honor Awards:

H. Caldwell - PHS Superior Service Award

R. Morrison - NIH Award of Merit

Journal Editorial Boards:

H. Caldwell - Infection and Immunity (Editorial Board)
Cell (Invited Reviewer)
Journal of Bacteriology (Invited Reviewer)
Journal of Infectious Diseases (Invited Reviewer)
Journal of Immunology (Invited Reviewer)
Proceedings of the National Academy of Sciences of (Invited Reviewer)
Microbiol Pathogenesis (Invited Reviewer)
Journal of General Microbiology (Invited Reviewer)

R. Morrison - Infection and Immunity (Invited Reviewer)
Journal Infectious Diseases (Invited Reviewer)
Journal of Immunology (Invited Reviewer)

T. Hackstadt - Journal of Bacteriology (Invited Reviewer)
Molecular Microbiology (Invited Reviewer)
Journal of Leukocyte Biology (Invited Reviewer)

Professional Posts:

H. Caldwell - Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.

R. Morrison - Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.

Elected Post:

T. Hackstadt - Councilor-at-large, American Society for Rickettsia and Rickettsial Diseases.

Invited Lectures and Participation in Meetings and Symposia:

S. Awasthi -	American Society of Microbiology, New Orleans, Louisiana American Society for Rickettsiology and Rickettsial Diseases, Hamilton, Montana
C. Barry -	American Society of Microbiology, New Orleans, Louisiana Fallen Leaf Lake Conference, Bacterial Virulence Mechanisms, Fallen Leaf Lake, South Tahoe, California
T. Brickman -	American Society of Microbiology, New Orleans, Louisiana
H. Caldwell -	Trachoma Research Meeting at the Marine Biological Laboratory in Wood's Hole.
R. Gilmore -	American Society of Microbiology, New Orleans, Louisiana American Society for Rickettsiology and Rickettsial Diseases, Hamilton, Montana
T. Hackstadt -	American Society of Microbiology, New Orleans, Louisiana American Society for Rickettsiology and Rickettsial Diseases, Hamilton, Montana National Symposium on Safety in the Biomedical Laboratory, Atlanta, Georgia
R. Heinzen -	American Society of Microbiology, New Orleans, Louisiana
R. Morrison -	National Institute of Allergy and Infectious Diseases, NIH Research Day, Molecular Pathogenesis Workshop, Bethesda, MD. (1992) The Interplay of Microbes and Their Hosts, RML, Hamilton, Montana (1992) Interscience conference on Antimicrobial Agents and Chemotherapy
P. Policastro -	American Society for Microbiology, New Orleans, Louisiana American Society for Rickettsiology and Rickettsial Diseases, Hamilton, Montana
H. Su -	How to make Microcapsules, St. Louis, Missouri Modern Approaches to New Vaccines, Including Prevention of Aids, Cold Spring Harbor, New York

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 AI 00519-05- LICP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunopathogenesis of Chlamydial Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.P. Morrison	Microbiologist	LICP, NIAID
Others:	D.D. Rockey	IRTA	LICP, NIAID
	K. Lyng	Chemist	LICP, NIAID

COOPERATING UNITS *(if any)*
Univ. Toronto, Ontario, Canada (R. Inmam); Univ. Wisconsin Med. Sch., Madison, WI (G.Byrne); New York Hosp.-Cornell Med. Ctr., New York, NY (S. Witkin); University of Montana, Division of Biological Science, Missoula, Montana (Y. Ying).

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Immunobiology of Intracellular Parasites

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Chlamydia trachomatis is a bacterial pathogen that generally infects the ocular and genital mucosal epithelium and produces uncomplicated infections such as conjunctivitis, urethritis, and cervicitis. However, these can progress to chronic infections that provoke severe inflammatory responses that lead to blindness and infertility. The objectives of this project are to characterize the antigenic properties of the chlamydial 60kDa heat shock protein (hsp60), which has previously been shown to stimulate immune response responses that contribute to the pathology of chlamydial disease, and to use in vitro models of chlamydial infection to study chlamydial persistence.

The antigenic structure of the hsp60 was characterized by defining immunogenic regions of the polypeptide using monoclonal antibodies and sera from patients having chlamydial infection. Ten anti-chlamydial hsp60 mAbs were prepared and their reactive epitopes precisely mapped. Most mAbs reacted with linear epitopes located at the amino or carboxyl termini of the hsp60 polypeptide. The mAbs were useful for detection of chlamydial inclusions by indirect immunofluorescence, and for the purification of recombinant hsp60 by immunoaffinity chromatography. Patients with post-chlamydial reactive arthritis and women having had ectopic pregnancies had high titered anti-hsp60 serum antibody. The anti-hsp60 response was predominantly chlamydial specific (i.e. did not crossreact with the *E. coli* hsp60 homologue) and was localized to the amino and carboxyl termini, and to a polypeptide containing amino acids 200 to 300. Further analyses are being performed to determine if the anti-hsp60 response differs among patients with acute or chronic chlamydial disease.

The antigenic stimulus for the chronic inflammation associated with blindness and infertility is unknown, but could result from persistent infection. To determine factors that can lead to persistent chlamydial infection we used an in vitro model of chlamydial infection. Interferon-γ (IFN-γ), a cytokine released from lymphocytes in response to antigenic stimulation, was found to cause persistent chlamydial infection in vitro. IFN-γ inhibited the production of infectious progeny, despite the presence of intracellular chlamydiae. Analysis of infected cells treated with IFN-γ revealed morphological changes in chlamydiae characterized large abnormal intracellular forms. These atypical forms were found to exhibit differential expression of chlamydial antigens. The chlamydial hsp60 was found to be in abundance, while only a paucity of the major outer membrane protein (MOMP) was detected. Despite altered morphology and antigen expression, infectious progeny were recoverable when IFN-γ was removed. If IFN-γ has a similar effect in vivo, then persistently infected cells containing an abundance of hsp60, a deleterious immune target, and a paucity of MOMP, a protective immune target, could serve as depots of antigen for the stimulation of chronic inflammation that precedes blindness or infertility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00567-02 LICP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT ***(80 characters or less. Title must fit on one line between the borders.)***
Rickettsial Surface Structure and Function

PRINCIPAL INVESTIGATOR ***(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)***

PI:	T. Hackstadt	Expert	LICP, NIAID
Others:	P.F. Policastro	Senior Staff Fellow	LICP, NIAID
	R.D. Gilmore	Staff Fellow	LICP, NIAID
	R.A. Heinzen	IRTA	LICP, NIAID
	S. Awasthi	Visiting Fellow	LICP, NIAID
	R.E. Mann	Bio Lab Tech.	LICP, NIAID

COOPERATING UNITS ***(If any)***
NONE

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Host-Parasite Interactions Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

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  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK ***(Use standard unreduced type. Do not exceed the space provided..)***

Rickettsia rickettsii is the causative agent of Rocky Mountain spotted fever. Current incidence averages approximately 1000 individuals per year in the United States. Related members of the spotted fever group include the rickettsial agents of Mediterranean spotted fever, Australian tick typhus, North Asian tick typhus, and rickettsialpox as well as several non-pathogenic species. Other members of the genus *Rickettsia* are the etiologic agents of endemic and epidemic typhus.

Efforts have been focused on a pair of immunodominant surface protein antigens with estimated mol. masses of 190 and 135 kDa and termed rOmp A and B, respectively. These proteins are of interest due to their surface location, strong immunogenicity, and reactivity with monoclonal antibodies that protect mice against lethal rickettsial challenge. Our current objectives are to determine the role of these proteins in rickettsial interaction with the host cell. These studies involve characterization of the variability in both protein and gene structure between species of rickettsiae that differ in virulence both in animal models and cell culture. In the past year we have shown that the rOmp B protein is encoded by an unusually large open reading frame and then processed to yield stable membrane associated products of 135 and 32 kDa. A spontaneously occurring mutant that is unable to complete this processing step is avirulent. The rOmp A protein, a protective immunogen, has 13 direct repeat units of 72 - 75 amino acids each that, although similar, can be categorized as type I or II repeats. DNA sequence analysis of the rOmp A protein from diverse species of rickettsiae indicates variation in the order of the repeat units but little sequence divergence between corresponding type I or II units. In addition, we are initiating studies to determine the host cells response to infection by intracellular parasites. Host cell responses so far detected include changes in actin structure possibly mediated by the parasite and aspects of signal transduction such as phospholipase C activity, inositol phosphate production, and protein kinase C activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00612-02-LICP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Analysis of Recombinant Chlamydial Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Scott Manning	Staff Fellow	LICP, NIAID
Others:			

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Immunobiology of Intracellular Parasites

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The focus of this project is the use of *E. coli* to express the *Chlamydia trachomatis* major outer membrane protein (MOMP) for structural, functional and immunological analyses. MOMP induces protective immune responses and is believed to serve several important biological functions including acting as an adhesin. The immediate goals of this project are two-fold: first, to evaluate recombinant MOMP expressed in *E. coli* as an accurate and valid model for the native molecule and second, to express defined MOMP epitopes as fusions with immunologically stimulatory proteins.

Expression of the MOMP gene (omp1) in *E. coli* resulted in signal peptide cleavage and the production of molecule with the correct molecular weight. The expression of this molecule was, however, extremely toxic to the host cells and neither surface localization nor the presence of conformational epitopes could be demonstrated. These results suggest that MOMP expressed in *E. coli* does not assume a native, surface localized, conformation and thus is of little utility as a structural or functional model; nor as a source for conformationally correct vaccine antigen.

Defined B- and T-Cell MOMP epitopes have been expressed as fusions with either the A or B subunits of *E. coli* heat labile toxin LT. Chlamydia are primarily pathogens of mucosal surfaces and a successful vaccine will require the induction of a strong mucosal immune response. *E. coli* LT is a potent stimulator of mucosal immunity. MOMP/LT fusion may, therefore, be capable of stimulating strong anti-chlamydial immune responses. Genetic constructions which express MOMP/LT chimeras have been completed and characterization of the structural and functional properties of these molecules is ongoing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 AI 00631-01 LICP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Biology of Intracellular Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Hackstadt	Expert	LICP, NIAID
Others:	C.E. Barry	IRTA	LICP, NIAID
	T. Brickman	IRTA	LICP, NIAID
	J.D. Sager	Bio. Lab. Tech.	LICP, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION
Immunobiology of Intracellular Parasites

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Chlamydia trachomatis is the etiologic agent of a diverse group of clinical syndromes including trachoma, a chronic inflammatory disease of the eye, and sexually transmitted diseases.

Chlamydiae are bacterial obligate intracellular parasites that undergo a unique biphasic life cycle involving cell types adapted for extracellular survival and dispersal and for intracellular growth. The regulatory mechanisms governing this complex life cycle are poorly understood. We have characterized a pair of unique proteins with homology to eukaryotic histone H1. Histone H1 homologs are rare among prokaryotes. *C. trachomatis* is unusual in that it possesses two histone analogs. The genes are expressed only during the late stages of the chlamydial life cycle concomitant with the reorganization of chlamydial reticulate bodies into elementary bodies suggesting that the histone analogs play a role in the condensation of chlamydial chromatin during intracellular differentiation. Expression of the 18 kDa histone analog, Hc1, in recombinant hosts induces a compaction of the bacterial chromatin that ultrastructurally resembles events late in the chlamydial developmental cycle. The Hc1 protein therefore appears to play a major role in the condensation of the chlamydial nucleoid characteristic of mature EBs and in doing so contributes to an overall down regulation of gene expression. Effects on DNA structure may play a significant role in the regulation of gene expression during chlamydial development. Understanding the controlling mechanisms for gene expression and differentiation may suggest unique methods of interrupting parasite replication.

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Summary Statement
LABORATORY OF INFECTIOUS DISEASES
National Institute of Allergy and Infectious Diseases
October 1, 1991 to September 30, 1992

HEPATITIS VIRUSES SECTION

Recent successes achieved by scientists in the Section include the development of a cell culture-adapted, attenuated mutant of HAV and identification of the genetic basis for these two phenotypes. Initially, full length RNA transcripts of full length cDNAs were constructed from the single strand, positive sense RNA genome of parental wild type HAV and its attenuated HAV mutant. Full length RNA transcripts of cDNAs were then shown to be infectious for cell culture. This made it possible to construct chimeric viruses and to introduce site-specific mutations into the HAV genome. By using these strategies it was possible to identify the mutations in the cell culture-adapted mutant responsible for its efficient replication in tissue culture as well as its attenuation for experimental non-human primates. The attenuated HAV mutant is currently in clinical trial.

Sequence analysis of an unusual HAV, originally recovered from an ill monkey in Moscow, revealed it to be the most divergent strain studied thus far. In addition, the simian isolate grew efficiently in monkey cell cultures that barely supported the growth of human HAV strains. The host range of the simian isolate was also of some interest because it caused hepatitis in African green monkeys and marmosets, but it was avirulent in chimpanzees. This unusual host range suggested that the simian HAV isolate might be attenuated for humans. If this be the case, it may be possible to use the strain for "Jennerian" immunization of humans because experimental infection of chimpanzees induced resistance to subsequent challenge with virulent human HAV.

The woodchuck hepatitis virus (WHV) is taxonomically and antigenically related to human hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations are etiological in nature. Development of a method for infecting woodchucks by direct inoculation of full length WHV DNA into the liver now makes it possible to study the effect of site-specific mutations upon various functions and properties of the virus in its natural host. This unusual system for performing mutational analysis should continue to yield important dividends in the future, including identification of viral determinants of oncogenesis which are critical to the development of hepatocellular carcinoma, an event that occurs with unusually high frequency (100%) during persistent WHV infection of woodchucks.

Finally, the cloning and sequencing of hepatitis C virus (HCV) by others has allowed members of the Section to revisit this important hepatic pathogen originally identified in LID twenty years ago. Studies performed with newly developed molecular tools have yielded a number of surprises. For example, the virus was shown to exhibit a greater than expected degree of genetic heterogeneity. This property of the virus has frustrated the initial attempts to develop a sensitive, specific laboratory test for detection of virus. The only practical method for identification of virus infection is PCR. However, sequence heterogeneity has caused a high rate of false negative PCR results. As a consequence, scientists in the Section sought PCR primers that would yield the lowest possible rate of false negative results. When sequences of

HCV strains characterized in the Section and in other laboratories were compared, two highly conserved domains were identified within the 5' non-coding region of the viral genome and these domains were used to prepare a new set of PCR primers. The new primers detected every virus strain that had been identified by other primer sets without yielding false positive results.

In addition, it was observed that infection of chimpanzees failed to induce resistance to infection when animals were rechallenged with the same strain or a related strain of HCV. However, prior infection with HCV induced partial protection against hepatitis following rechallenge. At this time it is not clear whether HCV infection of humans induces an immune response that confers complete resistance to subsequent HCV infection and disease or for that matter whether such a response can be induced by immunization. Formulation of a successful strategy for immunoprophylaxis awaits resolution of these issues.

GASTROENTERITIS VIRUSES PROGRAM (EPIDEMIOLOGY SECTION)

The "Jennerian" approach to immunization has been successfully applied to the development of a promising candidate live rotavirus vaccine. A simian rotavirus derived from rhesus monkeys, designated rhesus rotavirus (RRV), was developed in LID as a surrogate strain for immunization against rotavirus disease of infants and small children. This simian virus is attenuated in young infants and is closely related antigenically to serotype 3 human rotaviruses which constitute one of the 4 medically important serotypes. In addition, a modified "Jennerian" approach was pursued in order to expand the antigenic repertoire of the vaccine to include the major viral outer capsid protective antigen (VP7) of each of the human rotaviruses of major medical importance (*i.e.*, serotypes 1, 2, 3 and 4). This approach involved the construction of single gene reassortant rotaviruses that contained a human rotavirus VP7 gene that conferred antigenic specificity of serotype 1, 2 or 4, while the remaining 10 genes were derived from RRV. To date, impressive resistance has been documented in some vaccine trials, but not in others. During the past year several reasons for vaccine failure were identified and appropriate action was taken to increase efficacy of vaccine for infants and young children. At present, a quadrivalent RRV vaccine (serotypes 1, 2, 3 and 4) is being tested in a number of clinical trials throughout the world and the results that have become available are most encouraging.

A major goal of the rotavirus molecular biology program has been the development of a successful strategy for introduction of site-specific mutations into the rotavirus genome. Recently, an encouraging start was made in this direction. In these studies, cDNA clones encoding plus- or minus-sense analogs of the rotavirus VP7 gene were constructed. These analogs contained the bacterial chloramphenicol acetyl transferase (CAT) open reading frame, in either plus or minus sense, flanked by the 5' and 3' noncoding regions of the plus or minus RNA strand of the VP7 gene. When transfected into cells and complemented by infection with homologous or closely related heterologous strains of rotavirus, the synthetic plus sense RNAs were replicated, transcribed and expressed. This provides a new, sensitive system for characterizing rotavirus gene replication and expression. For example, it was possible to map *cis*-acting nucleotide sequences at the 3' end of the plus strand that were required for amplification of the input synthetic RNA.

Another important goal of the program has been the identification of genetic determinants of virulence of a pathogenic rotavirus for its natural host. Studies were performed in newborn gnotobiotic piglets which develop diarrhea when infected with the SB-1A strain of porcine rotavirus. In contrast, the DS-1 strain of human rotavirus does not initiate infection or cause diarrhea in these animals. After considerable effort scientists in the Epidemiology Section succeeded in preparing single gene substitution rotavirus reassortants that derived their 1st, 2nd, 5th, 6th, 7th, 8th, or 11th gene from the DS-1 human rotavirus, while the remaining 10 genes were derived from the SB-1A porcine rotavirus. These reassortant viruses infected piglets and caused diarrhea. However, similar single gene reassortant viruses that derived their 3rd, 4th, 9th, or 10th gene from the DS-1 strain did not infect piglets or produce disease. Thus, 4 rotavirus genes were shown to play a separate and essential role in virulence of a porcine rotavirus for its natural host.

RESPIRATORY VIRUSES SECTION

Twenty-five years ago our initial attempts to develop vaccines for important pediatric respiratory tract viral pathogens involved the development of formalin-inactivated respiratory syncytial virus (RSV) and parainfluenza virus vaccines modeled after the successful, licensed inactivated influenza virus vaccine. However, in clinical trials, these paramyxovirus vaccines did not prevent RSV or parainfluenza virus infection. Even more unexpected was the potentiation of RSV disease that occurred when recipients of inactivated RSV vaccine subsequently underwent natural infection with the virus.

An understanding of disease potentiation awaited the recent development of RSV antigen-specific antibody assays and an experimental animal model in which the phenomenon could be reproduced. Through the sustained efforts of the Respiratory Viruses Section an explanation of potentiation was finally achieved. Briefly, the inactivated RSV vaccine was shown to create a series of immunological imbalances. There was an imbalance between systemic and local secretory antibody responses. Thus, vaccine induced a high level of serum RSV F glycoprotein antibodies, but, based on experience with other related viruses, it is likely that very little local secretory antibody was induced. An imbalance was also created within the serum antibody response. Abundant antibodies to RSV F glycoprotein were induced but these immunoglobulins had very low neutralizing activity. Also, the response to G, the other major RSV envelope glycoprotein, was low in infants. Finally, an imbalance was identified within the cell-mediated immune response. Inactivated RSV vaccine induced an exaggerated lymphocyte proliferative responsive to RSV antigens, probably mediated by T4 lymphocytes. In contrast, inactivated vaccine did not induce a RSV-specific T8 CTL response. As a consequence of these imbalances, vaccinees were susceptible to RSV infection because inactivated vaccine did not induce an adequate secretory or serum neutralizing antibody response. When infection occurred, virus replication was not effectively restrained because RSV serum neutralizing antibodies were insufficient as was the RSV T8 CTL response. However, RSV T4 memory cells were present in high concentration and able to undergo amplification and expression of deleterious effector functions such as delayed hypersensitivity. In addition, vaccinees failed to develop a normal level of neutralizing antibodies in response to RSV infection. This reduced neutralizing antibody response may have delayed resolution of infection which could contribute to the increased severity of disease. The reduced neutralizing antibody response to infection was probably the result of immunosuppression by abundant non-neutralizing RSV antibodies and/or repertoire bias. Each of these forms of imbalance can now be assayed for early in the course of vaccine development. In fact, such an evaluation of purified RSV F and chimeric F/G glycoprotein candidate vaccines prepared by others, led us to suspend our pre-clinical study of these materials.

The development of temperature-sensitive (*ts*) and cold passage-attenuated (*ca*) mutants of RSV was pursued concurrent with our investigation of disease potentiation by inactivated RSV vaccine. Although the *ts*-1 and *ca* mutants that were developed 15-20 years ago were both overattenuated for adults, these mutants were satisfactorily attenuated and immunogenic in young seropositive children. However, both mutants retained a low level of residual virulence for the upper respiratory tract of seronegative infants. In addition, the *ts*-1 mutant exhibited evidence of genetic instability, particularly in seronegative infants. However, ts^+ "revertants" constituted less than 0.01% of virus shed by young vaccinees and the "revertants" did not take over and become dominant. Also, the presence of this small concentration of ts^+ virus did not appear to be related to the symptoms of mild upper respiratory tract involvement.

The decision 12 years ago to: (i) terminate clinical evaluation of the *ts*-1 mutant and (ii) not to proceed to clinical evaluation of the remutagenized derivatives of *ts*-1, *i.e.*, *ts*-1 NG-1, and *ts*-1 NG-16, may not have been warranted. In retrospect, the requirement for complete genetic stability of *ts* mutant vaccine strains appears to have been an unrealistic goal, especially in the case of single strand RNA viruses whose viral polymerase lacks a proof reading function. Unfortunately, this requirement precluded further evaluation of RSV mutants that exhibited any evidence of genetic instability. At that time, it was not known that live poliovirus vaccine strains undergo mutation with very high frequency without causing disease. For example, one of the two major mutations responsible for the greatly reduced neurovirulence of the type 3 vaccine strain for monkeys, a base substitution at position 472 in the 5' noncoding region of the viral genome, regularly reverts to wild type sequence early during infection, but the risk of paralytic disease following immunization is very low ($\sim 10^{-6}$). A similar restoration of wild type sequence at the site of the corresponding mutation in type 1 poliovirus vaccine strain also occurs in ~50% of immunizations. Thus, it is probably unwise to reject candidate vaccine strains without first determining the clinical significance and acceptability of genetic changes that occur during immunization.

A recent re-evaluation of the *ts*-1 and *ts*-1 NG-1 mutants and another *ts* mutant (*ts*-4) indicated that none of these mutants was able to replicate significantly in the lower respiratory tract of the chimpanzee, confirming our original judgement that these mutants did not pose a threat to the lungs. For this reason, we have sought to introduce additional mutations into the *ts*-1 and *ca* mutants because they were already highly attenuated for susceptible infants and required only the loss of a low level of residual virulence for the upper respiratory tract to be considered satisfactory for use in young infants. Based upon these considerations, the development of live attenuated RSV vaccine strains has been resumed with full vigor. The cold passaged mutant is being subjected to additional passages at even lower temperature and, independently, *ts* mutations are being introduced. Similarly, the *ts* mutants are being subjected to cold passage at the lowest possible temperature.

Progress has also been made in the development of a live attenuated parainfluenza type 3 virus (PIV3) vaccine. Cold-adapted (*ca*) mutants developed by Dr. Belshe, a former Research Associate in LID, appear to be promising based upon our studies in non-human primates and collaborative studies involving seronegative infants and young children. In addition, the "Jennerian approach" to immunization shows promise. This approach involves the use of bovine PIV3, which is related antigenically to human PIV3. Initially it was shown that this bovine virus is restricted in its replication in the lungs of non-human primates. More recently, several seronegative infants who received 10^4 or 10^5 $TCID_{50}$ of bovine PIV3 by the intranasal route shed virus and developed antibodies to human PIV3, but did not become ill.

Our molecular biologic studies of RSV over the past 6 years have defined the RSV genome with respect to gene order, gene expression, and control of transcription and replication. In addition, a thorough description of gene products, their intracellular processing, immunogenicity and role in protective immunity have set the scene for evaluation of recombinant viral vector-based immunization strategies. Interesting observations were made confirming the primacy of the RSV viral surface glycoproteins, F and G, in protective immunity. Unfortunately, none of the vaccinia virus- or adenovirus-RSV recombinants induced impressive resistance in chimpanzees. However, infection with the former recombinants primed chimpanzees so that subsequent RSV infection induced a booster response that attained the highest serum neutralizing antibody titers ever registered in our laboratory. Perhaps, this priming effect could prove useful in a combined strategy that may be required to overcome the immunosuppressive effect of maternally-derived antibodies possessed by all young infants.

Until now it has not been possible to rescue live RSV from cloned complementary DNA (cDNA). Achievement of this goal is complicated by the fact that RSV belongs to a class of viruses (non-segmented, negative strand RNA viruses) whose genetic material (viral RNA genome) is not infectious. Viral RNA, whether intracellular or present in virions, is always tightly encapsidated in a nucleocapsid which contains viral proteins required for transcription and replication. Thus, the nucleocapsid probably represents the minimum unit of infectivity. A promising strategy to overcome this barrier was developed during the past year. First, the RSV genetic sequences that direct transcription and replication of viral RNA were identified and used to construct a "mini" viral RNA bearing a single marker gene (CAT) flanked by the RSV regulatory signals. Introduction of this mini-RNA into cells infected with RSV resulted in efficient amplification of mini-RNAs and expression of CAT protein. The "mini" viral RNAs were also packaged efficiently into virus particles. The nucleocapsid proteins required for these activities were provided by the RS helper virus. Subsequently, it should be possible to rescue live RSV from full length RNA transcripts prepared from full length RSV cDNA. The benefits of such an RSV rescue system should be manifold because it will allow us to directly identify attenuating mutations in existing candidate vaccine strains, introduce specific mutations into RSV for molecular and genetic studies, and produce a new generation of effective, more stable live attenuated vaccine virus strains.

FLAVIVIRUS MOLECULAR BIOLOGY PROGRAM (MOLECULAR VIRAL BIOLOGY SECTION)

Molecular biological studies have yielded the complete sequence of the non-segmented positive strand RNA genome of dengue type 4 virus. The viral genome was shown to have a single long open reading frame that encoded a single long viral polyprotein containing the three structural proteins, C, pre-M and E in this order, at the 5' terminal position followed by 7 non-structural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5. Concurrently, the strategy used by the virus to process the viral polyprotein into its constituent proteins was defined in considerable detail including the identification and characterization of cleavage sites and the cellular and viral proteases responsible for specific cleavages. In addition, individual genes or combinations of genes were expressed by vaccinia virus or baculovirus recombinants. In this manner, the viral envelope glycoprotein (E), pre membrane protein (pre-M) and non-structural protein one (NS1) were identified as independent protective antigens.

One of the initial goals of the dengue virus program was the construction of full length viral cDNA that could serve as a template for full length RNA transcripts that are infectious for susceptible cells in culture. After 4 years of intensive effort a functional full-length dengue virus cDNA was produced. Full-length RNA transcripts of this template were shown to be infectious for cells in culture. As a consequence, it became possible for the first time to introduce mutations into full-length dengue cDNA by site-directed mutagenesis and subsequently recover viable dengue virus bearing these mutations. Viral mutants produced in this manner should prove useful for identifying regulatory elements involved in transcription, replication and packaging of viral RNA and for elucidating the mechanisms involved in processing the single long viral polyprotein into its discrete proteins.

Another immediate application of full-length, functional dengue cDNA is in the area of vaccine development because viral progeny of the infectious RNA transcripts were recently shown to be infectious for monkeys. Restriction of dengue virus replication should cause attenuation for primates, however, it is essential that a proper balance between attenuation and immunogenicity be achieved so that an immunizing infection can occur without the development of undesirable signs or symptoms of disease. The identification of dengue virus mutants with this desirable balance of attenuation and immunogenicity is an empirical process and may require the evaluation of a large number of mutants. Scientists in the Section have already constructed a viable mutant that is restricted in virus replication as a consequence of amino acid substitution in the viral polyprotein at the cleavage site between dengue virus non-structural proteins NS1 and NS2a. Certain amino acid substitutions in this region decrease the efficiency of cleavage of NS1 and NS2a, thereby decreasing the overall processing of the viral polyprotein. Similarly, it has been possible to create viable deletions in the 5' or 3' non-coding region of the viral genome that restrict virus replication. Such deletion mutants offer the theoretical advantage of being less subject to reversion of phenotype than amino acid substitution mutants.

In addition, viable chimeric intertypic dengue viruses were produced by replacement of the structural protein genes of the dengue type 4 virus cDNA clone with the corresponding genes of type 1 or type 2 dengue virus. The dengue type 2 virus used for this purpose was a mouse neurovirulent mutant (New Guinea C strain). The dengue type 4/dengue type 2 virus chimera retained much of the neurovirulence of its type 2 virus parent, suggesting that most of the genetic determinants of mouse neurovirulence map within the three structural genes.

It is likely that there will be a requirement for inclusion of all four serotypes in a live attenuated dengue virus vaccine in order to induce broad immunity that would preclude the development of dengue hemorrhagic fever/shock syndrome. This severe disease is thought to be caused in part by secondary virus infection of individuals who possess heterotypic, non-protective dengue antibodies. The simplest strategy to achieve the goal of broad resistance is to identify viable attenuating mutations that can be introduced into the non-coding regions and/or the non-structural protein genes of the dengue type 4 virus. After this has been accomplished, cDNA of the suitably attenuated type 4 virus could be used to produce a type 1, type 2 or type 3 chimera which exhibits the serotype specificity of the type 1, type 2 or type 3 parent. A similar strategy might also be possible for other flaviviruses that are more distantly related to dengue type 4 virus. The feasibility of this approach was suggested by the successful construction of a viable dengue type 4 virus/tick-borne encephalitis (TBE) virus chimera that possessed the membrane (M) and envelope glycoprotein (E) genes of the latter virus. The dengue/TBE (ME) chimera grows to higher titer in simian cell culture than its dengue virus parent. In addition, the chimeric virus retains much of the neurovirulence of TBE virus that is evident following direct inoculation into the brain, but the chimera lacks the peripheral invasiveness of its TBE virus parent. Significantly, mice infected by the intraperitoneal (IP) or intradermal (ID) route with the dengue/TBE virus chimera survived without symptoms and were completely resistant to a subsequent lethal IP challenge with TBE virus. The observation that the chimera lacks the peripheral invasiveness of its TBE virus parent suggests that it may be possible to use such a chimera, suitably modified to abrogate direct neurovirulence for the brain, to develop a live attenuated virus vaccine for prevention of illness caused by TBE virus. This tick-borne virus is among the most virulent of all flaviviruses (mortality ~30%) and currently, effective control measures are not generally available.

IMMUNODEFICIENCY SYNDROMES PRODUCED BY LENTIVIRUSES INDIGENOUS TO MONKEYS AND CATS (IMMUNODEFICIENCY VIRUSES SECTION)

Five years ago a new program was initiated for the study of immunodeficiency syndromes produced in monkeys and cats by lentiviruses indigenous to these species. Two experimental systems were established: (1) an immune deficiency syndrome in macaques caused by the simian immunodeficiency virus (SIV) derived from sooty mangabeys, *i.e.*, SIVsm; and (2) an immune deficiency syndrome in cats caused by a feline immunodeficiency virus (FIV). The syndromes produced by these viruses in their respective hosts resemble human AIDS and thus, SIV-infected monkeys and FIV-infected cats constitute experimental surrogates for human AIDS. Molecular clones of both SIVsm and FIV were constructed and sequenced and shown to be infectious for cell culture and the natural host. Consistent with the view that the SIVsm system is a potentially useful experimental model for AIDS was the observation that SIVsm is more closely related in sequence to human immune deficiency virus type 2 (HIV-2) than any other known non-human primate lentivirus. However, SIVsm is more distantly related to HIV-1 as is the case for other simian lentiviruses. Scientists in the Section have also characterized related lentiviruses from other African primates such as African green monkeys (SIVagm) and Sykes' monkeys (SIVsyk).

Recently, the determinants of pathogenesis of an acutely lethal SIV mutant were studied in collaboration with the Yerkes Primate Center. This mutant of SIVsm, designated SIVsm PBj, causes a fatal syndrome of fever, massive diarrhea and acidosis resulting in death by 4 to 14 days after infection. Six full-length molecular clones of SIVsm PBj were derived by PCR amplification and cloning. Two of these clones induced the acute fatal disease. Chimeras constructed between an acutely lethal PBj clone and its SIVsm parent demonstrated that genetic determinants of this phenotype are present in both the 3' and 5' halves of the genome. In addition, these studies suggested that one or more of the genetic determinants of virulence are present in the SIVsm PBj envelope gene. Finally, the SIVsm PBj LTR (with its duplicated core enhancer) does not appear to play a role in the induction of acute disease. In future studies an attempt will be made to define the pathogenic mechanisms of SIVsm PBj-induced disease, and the role of cytokines in this syndrome. Full length clones of SIVsm that will reproducibly induce immunodeficiency with a short latency (~3 to 6 months) are also being generated. These clones will allow us to: (i) dissect the viral determinants of immunodeficiency more efficiently and (ii) examine the role of some of the nonessential SIV genes such as vpx, vpr and nef.

Our first effort to develop a concentrated, inactivated, whole virion SIV vaccine was highly successful. Macaque monkeys immunized with psoralen-inactivated whole SIV developed a robust SIV neutralizing antibody response and were resistant to intravenous challenge with fifty monkey infectious doses$_{50}$ (MID$_{50}$) of cell-free virus of an heterologous, divergent SIV strain (20% envelope glycoprotein amino acid sequence divergence from the vaccine strain) as well as the homologous SIV strain. This vaccine trial is the first to demonstrate such broad protection using a molecular clone as the vaccine virus.

Plasma from immunized, protected macaques (described above) was pooled and the IgG fraction was isolated by ion-exchange chromatography. IgG purified from uninfected macaques served as a control. Four macaques received an intravenous infusion of 50 mg/kg of SIV IgG and two macaques received an equivalent amount of normal IgG. Twenty-four hours after infusion, macaques were challenged intravenously with 50 MID_{50} of cell-free SIVsm (the homologous challenge described above). Whereas, both control animals became infected by 2 weeks after challenge, the four SIV IgG recipients remained free of virus (as indicated by negative virus isolation attempts and PCR). This study demonstrated that the protective effect induced by vaccination with inactivated SIV was mediated solely or in part by IgG. It should be noted that the recipients of IgG from immunized monkeys achieved a serum neutralizing antibody titer for SIV that was one-fourth that of the donor monkeys.

Subsequently, five immunized macaques that resisted challenge with cell-free SIV were boosted with the inactivated SIV vaccine and re-challenged 4 weeks later with 50 MID_{50} of cell-associated SIV. Both control animals and all five vaccinees became infected despite the fact that the vaccinated animals had a high level of serum neutralizing activity against the challenge virus. These macaques are being monitored for differences in disease course that might suggest protection from disease. Eight months post-challenge, all five immunized macaques are alive, whereas four of six control macaques which received a similar dose of SIVsm have died as a result of immunodeficiency. Other vaccine-related activities include the construction and characterization of integrase deletion and/or packaging mutants of SIV.

Issues in immunoprophylaxis that will be addressed in future studies include the feasibility of increasing vaccine efficacy by priming monkeys and subsequently boosting their level of resistance and duration of immunity. It may be possible to achieve this goal by initially infecting monkeys with a recombinant vector expressing SIV envelope and later boosting their resistance by inoculation of purified SIV envelope or inactivated virus. The reverse sequence will also be evaluated. Of considerable concern is the possibility that the psoralen-inactivated SIV vaccine produced its protective effect by stimulating an antibody response to virus-associated antigens derived from the human cell line used to produce both the vaccine and the virus suspensions used to challenge vaccinated monkeys. This issue will be evaluated by: (1) absorbing anti-human cell antibodies from the serum of immunized monkeys and comparing the protective effect of the depleted product with that of unabsorbed serum, and (2) preparing vaccines and challenge virus suspensions in cell substrates that significantly reduce the possibility that an anti-host cell antigen response could contribute to vaccine-induced resistance.

HONORS AND AWARDS

Robert M. Chanock, M.D.

Member, Nominating Committee for ICN International Prize for Virology. 1991 Award Recipient, Wolfgang K. Joklik.

Invited speaker, Pathogenesis Symposium, The Scripps Research Institute, December 5, 1991.

Member, National Academy of Sciences Committee on International Security and Arms Control Working Group on Biological Weapons Control.

Member, Board of Scientific Governors of The Scripps Research Institute.

Co-organizer, Wallace P. Rowe Eighth Annual Symposium on Animal Virology, February 3-4, 1992.

Invited speaker, NIAID Grand Rounds, March 18, 1992.

Invited speaker, Pediatric Grand Rounds, Francis Scott Key Medical Center, May 26, 1992, "Respiratory Syncytial Virus Had Its Origin at Francis Scott Key Medical Center."

Invited participant at Conference,entitled "Molecular Mechanisms of Viral Latent Infections," Banbury Center at Cold Spring Harbor Laboratory, July 6-9, 1992.

Co-organizer, Modern Approaches to New Vaccines Including the Prevention of AIDS Conference, Cold Spring Harbor Laboratory, New York, September 16-20, 1992.

Albert Z. Kapikian, M.D.

Invited speaker, 8th International Congress of Virology, August 26-31, 1990, Berlin, Germany.

Invited chairman of session at meeting, "Modern Approaches to New Vaccines Including Prevention of AIDS," September 12-16, 1990, Cold Spring Harbor, New York.

Invited member, co-chairman of session, and invited speaker at 24th Joint Viral Diseases Panel Meeting of the Japan-U.S. Cooperative Medical Sciences Program, Sendai, Japan, September 27-30, 1990.

Invited speaker at symposium, "Immunology of Milk and the Neonate," Miami, Florida, October 14-17, 1990.

Invited chairman of session at Fourth NIH Rotavirus Vaccine Workshop at NIH, Bethesda, Maryland, November 5-6, 1990.

Invited speaker at dinner honoring Dr. Chanock who was a recipient of 1990 ICN International Prize in Virology at the Cloister, NIH Campus, February 7, 1991.

Invited speaker at 17th Pacific Science Congress, May 27-June 2, 1991, Honolulu, Hawaii

Invited speaker at a seminar at University of Tampere, Tampere, Finland, September 12, 1991.

Invited speaker at meeting, "Genetically Engineered Vaccines: Prospects for Oral Disease Prevention," November 6-8, 1991, NIH, Bethesda, Maryland.

Invited participant (and a speaker) at symposium, "27nm Viral Gastroenteritis Agents: Recent Progress," Stanford University, Palo Alto, California, November 8, 1991.

Invited speaker at NIH Clinical Center Grand Rounds, NIH, Bethesda, Maryland, December 4, 1991.

Invited speaker at Eighth Meeting of the Consultative Group for Vaccine Development of the AID/PHS Vaccine Development and Health Research Program (PASA), NIH, March 9-10, 1992.

Invited speaker at NIAID LCI Rounds, NIH, March 20, 1992.

Invited speaker at International Conference, "Vaccines for Enteric Diseases," April 13-15, 1992, Cambridge, England.

Invited speaker at First International Union of Biochemistry and Molecular Biology (IUBMB) Conference, "Biochemistry of Diseases," June 1-6, 1992, Nagoya, Japan.

Ching-Juh Lai, Ph.D.

Invited to participate in "Hepatitis C Virus and Related Viruses: Molecular Virology and Pathogenesis", Venice, Italy, July 4-16, 1992.

Brian R. Murphy, M.D.

Invited to participate as a World Health Organization Temporary Advisor and member of the Task Force on Measles Vaccine Development, Geneva, Switzerland, October 7, 1991.

Invited to participate in the Cold-Adapted Influenza Virus Vaccine Investigators' Meeting, Orlando, Florida, March 25-27, 1992.

Invited to speak at World Health Organization meeting, "Development of Vaccines Against Diseases Caused by Respiratory Syncytial Virus and Parainfluenza Type 3 Virus," April 21-22, 1992, Geneva, Switzerland.

Invited to participate in the Ninth Session of the Scientific Advisory Group of Experts (SAGE) of the Programme for Vaccine Development and Transdisease Vaccinology, Geneva, Switzerland, June 24-26, 1992.

Robert H. Purcell, M.D.

Invited speaker, Symposium on Chimpanzee Conservation and Public Health, Diagnon/Bioqual, Inc., Gaithersburg, MD, November 15-16, 1991.

Invited speaker, The Third International Symposium on Viral Hepatitis and Hepatocellular Carcinoma, Taipei, Taiwan, December 5-8, 1991.

Invited speaker, International Symposium on Hepatology, Kowloon, Hong Kong, December 13-15, 1991.

Invited speaker, Walter Reed Gastroenterology Symposium, Bethesda, MD, April 3-4, 1992.

Organizer and speaker (Annual Meeting), and Chairman, U.S. Delegation of the Hepatitis Panel, U.S.-Japan Cooperative Medical Science Program, Kofu, Japan, April 20-23, 1992.

Invited speaker, American Society for Microbiology Annual Meeting, Symposium on Hepatitis Viruses A-F, New Orleans, LA, May 26-30, 1992.

Invited speaker and recipient, Hans Popper Prize, Biennial Meeting of the International Association for the Study of the Liver, Brighton, England, June 3-6, 1992.

Co-organizer, chairman and invited speaker, Fourth International Symposium on Hepatitis Delta Virus and Liver Disease, Rhodes, Greece, June 8-10, 1992.

Invited speaker, International Symposium on Hepatitis A Virus Vaccine, Rhodes, Greece, June 10, 1992.

Mark Connors, M.D.

Invited to speak at World Health Organization meeting, "Development of Vaccines Against Diseases Caused by Respiratory Syncytial Virus and Parainfluenza Type 3 Virus," April 21-22, 1992, Geneva, Switzerland. Two presentations: "Development of RSV Subunit Vaccine" and "Cold-Adapted RSV Strains as Potential Vaccine."

James E. Crowe, Jr., M.D.

Invited to speak at World Health Organization meeting, "Development of Vaccines Against Diseases Caused by Respiratory Syncytial Virus and Parainfluenza Type 3 Virus," April 21-22, 1992, Geneva, Switzerland. Two presentations, "Testing of RSV Recombinant Vaccine in Animals (Chimpanzees)" and "Temperature-sensitive Mutants of RSV Virus as Potential Vaccine. "

Suzanne U. Emerson, Ph.D.

Invited speaker, Microbiology Department, The Mount Sinai Medical Center, New York, NY. "Use of infectious cDNA clones to study the molecular biology and pathogenicity of hepatitis A virus.", April 28, 1992.

Invited speaker, International Symposium on Active Immunization Against Hepatitis A, "Molecular basis of virulence and growth in cell culture," Vienna, Austria, January 28, 1992.

Patrizia Farci, M.D.

Invited speaker, American Society for the Study of Liver Diseases (AASLD), Chicago, IL. "Lack of protection against reinfection with hepatitis C virus (HCV) in multiple cross-challenges of chimpanzees," November 2-5, 1991.

Invited speaker to give a lecture for the Pediatric Grand Rounds at Memorial Sloan-Kettering Cancer Center, New York. "Hepatitis NANB in the 1990's," November 14, 1991.

Invited speaker, the Fourth International Symposium on Hepatitis Delta Virus and Liver Disease, Rhodes. "Treatment of chronic delta hepatitis with high and low doses of Interferon α-2a. A randomized controlled trial," June 8-10, 1992.

Kim Green, Ph.D.

Invited to participate in "Symposium on 27nm Gastroenteritis Viruses," Stanford University, November 8, 1991.

Susan Hall, Ph.D.

Invited to speak at World Health Organization meeting, "Development of Vaccines Against Diseases Caused by Respiratory Syncytial Virus and Parainfluenza Type 3 Virus," April 21-22, 1992, Geneva, Switzerland. Presentation, "Testing Cp Mutants of Parainfluenza Virus Type 3 in Animals (Monkey, Chimpanzee)."

Yasutaka Hoshino, D.V.M.

Invited speaker, U.S.-Japan Cooperative Medical Science Program, 25th Joint Working Conference on Viral Diseases, Charlottesville, VA, September 25, 1991. "Identification of genes involved in rotavirus virulence in a gnotobiotic piglet model."

Invited speaker, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, June 22, 1992. "Prospects for development of a rotavirus vaccine."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00308-07 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro and *In Vivo* Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS *(if any)*
Smith Kline RIT (d'Hondt); FDA (Daemer); Fairfield Hospital, Melbourne, Australia (Gust); AFIP (Ishak); WRAIR (Sjogren)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00311-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Search For New Hepatitis Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Patrizia Farci, M.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
Hospital for Sick Children, Toronto, Canada (Phillips); Howard University Medical School (Sewchand)

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SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.64	0.64	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1989 a possible paramyxovirus etiology for giant cell hepatitis was proposed, based on electron micrographic changes seen in the livers of patients with this disease. Collaborative studies, including attempts to transmit the disease to primates, are in progress.

Patients with thalassemia in Sardinia, Italy, receive monthly blood transfusions as therapy. Many of these patients have developed chronic hepatitis. Most cases could be identified as hepatitis B or hepatitis C, but some lacked markers of either of these viruses. These patients are being studied for evidence of previously unrecognized hepatitis viruses.

Patients with fulminant non-A, non-B hepatitis generally do not have evidence of infection with any recognized hepatitis virus. Only about 10% can be identified as having fulminant hepatitis C. The remaining 90% remain a diagnostic enigma and may be infected with one or more previously unrecognized viruses. In collaborative studies, we are attempting to transmit the disease to primates.

The objectives of this project are to identify and characterize new etiologic agents of hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A longer term objective is the development of passive and active immunoprophylaxis for these important human pathogens.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-938

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00314-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Woodchuck Virus: Molecular Biological Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roger Miller, Ph.D	Senior Staff Fellow	LID, NIAID
Others:	Hong-shu Chen, M.D.	Visiting Fellow	LID, NIAID
	Michael Kew, M.D., Ph.D.	Visiting Scientist	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
	Fumihiko Sugata, M.D.,D.M.Sci.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
Division of Molecular Virology & Immunology, Georgetown University, Washington, DC (Gerin); New York State College of Veterinary Medicine (Tennant)

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.17	0.33

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus, WHV infection of woodchucks provides a relevant and convenient model for understanding HBV infections of humans. The following experiments were performed: (1) detection of WHV mutants in chronically infected woodchucks, (2) mutant WHV genomes in serum were found to resemble rearranged viral integrants in hepatocellular carcinoma, (3) analysis of the X gene promoter, (4) newly identified open reading frames of WHV are not essential for virus replication, (5) mutational analysis of the WHV X gene, and (6) analysis of a WHV mutant with a defective precore gene.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00530-05 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular and Computer Analysis of the Hepatitis B Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Norio Ogata, M.D., Ph.D.	Visiting Scientist	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

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Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.8	PROFESSIONAL: 1.47	OTHER: 0.33

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis B virus (HBV) is the prototype member of the hepadnavirus family, which consists of hepatotropic viruses that infect at least six separate species. Infection with HBV causes polymorphic liver diseases including acute or fulminant hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Over 300 million people throughout the world are chronically infected with HBV and are at risk of developing hepatocellular carcinoma, one of the world's most prevalent forms of cancer. Recently, two classes of HBV mutants have been identified. The first is an antigenic variant of the virus surface (S) gene that was found in individuals who had received immunoprophylaxis for hepatitis B. In each case an identical single nucleotide substitution occurred that resulted in a single amino acid change in a loop of the S protein known to be important in neutralization of the virus and in stimulation of protective immunity. The emergence of this S-gene mutant form of HBV raises important questions about the significance of such neutralization escape mutants and the possible effect they may have on the efficacy of existing monoclonal (recombinant) vaccines for hepatitis B. The second HBV variant discovered recently has a single nucleotide substitution in the precore gene that results in the premature termination of this protein. This variant is associated with fulminant hepatitis in several regions of the world. The objectives of this study are to: (1) establish an animal model for the study of S-and precore gene mutants, (2) establish and determine the infectivity titer of a challenge pool of S-gene mutant virus, (3) determine the ability of licensed recombinant hepatitis B vaccines to protect chimpanzees against challenge with the S-gene mutant, (4) modify existing vaccines, if necessary, to increase their protective efficacy against S-gene mutants, and, (5) determine the complete nucleotide sequence of the precore variant.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-564-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00569-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Determination of Genetic Markers of Virulence & Adaptation to Cell-Culture of Hepatitis A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Sergei Tsarev, Ph.D., D.Sci.	Visiting Scientist	LID, NIAID
	Susan Gdovin, Ph.D.	IRTA Fellow	LID, NIAID
	Ann Funkhouser, M.D.	Research Associate (CO)	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

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Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.39	3.14	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis A virus (HAV) is a picornavirus with a single-stranded RNA genome of approximately 7500 nucleotides. The wild-type strain of HAV grows poorly in cell-culture, is not cytopathic, and yields a low titer of virus. A cell-culture adapted mutant has been isolated which grows significantly more efficiently in cell-culture and which is attenuated for marmosets and chimpanzees. The objectives of this project are to determine the genetic basis for virulence and adaptation to cell culture of HAV.

1. The importance of the 2B and 2C regions of the HAV genome for accelerated growth *in vitro* has been confirmed while a secondary role for the P3 region has been established.
2. New mutations have been identified in the 5' non-coding region which play a role in growth of a vaccine strain of HAV in a human lung cell line.
3. Infection of marmosets with chimeric viruses showed that multiple mutations are required for attenuation of virulence.
4. Two chimeric cDNA clones with genomic regions of the simian strain of HAV in the HM-175 strain background produced viable virus after transfection of cultured cells.
5. Clonal isolates of CV-1 cells were shown to differ greatly in their capacity to support HAV replication *in vitro*.

U.S. GOVERNMENT PRINTING OFFICE. 1991 0-654-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00570-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Hepatitis C Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
	Patrizia Farci, M.D.	Visiting Scientist	LID, NIAID
	Jens Bukh, M.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.97	0.83

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis C virus (HCV) is an important human pathogen that is strongly associated with transfusion related non-A, non-B (NANB) hepatitis. Amplification of HCV RNA sequences by reverse transcription and polymerase chain reaction (cDNA PCR) is the only practical method currently available to demonstrate viremia in patients with HCV infection. However, genetic heterogeneity among different HCV strains results in false negative results in cDNA PCR assays because of primer and template mismatch. HCV accounts for up to 25% of community-acquired hepatitis and over 90% of transfusion-associated hepatitis in the United States. The recent cloning and sequencing of the genome of HCV led to the development of specific serologic tests for antibody to HCV (anti-HCV) that utilized recombinant-derived viral antigens. These tests have been useful for diagnostic and screening purposes but they shed little light on immunity to HCV, in part because currently available antigens are expressed from parts of the HCV genome that encode nonstructural or internal structural proteins. Furthermore, the finding of genetic heterogeneity of the HCV genome, especially in the gene encoding the envelope proteins, suggests that there may be heterogeneity of viral envelope proteins similar to that seen in human immunodeficiency viruses. Such a finding would bode ill for attempts at vaccine development. The objectives of this project are to: (1) identify primer sets that have a low false negative rate in a cDNA PCR assay; (2) determine the nucleotide sequence of diverse HCV genomes; and (3) analyze whether infection of primates with HCV induces protective immunity.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00596-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vivo and *In Vitro* Studies of Hepatitis E Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Suzanne U. Emerson, Ph.D.	Microbiologist	LID, NIAID
Others:	Sergei Tsarev, Ph.D., D.Sci.	Visiting Fellow	LID, NIAID
	Shu-Rong Yin, M.D.	Visiting Fellow	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*
National Institute of Virology, Pune, India (Arankalle); All India Institute of Medical Research, New Delhi, India (Panda); Gene Labs, Inc., Redwood City, CA (Reyes); WRAIR, Washington, DC (Sjogren, Ticehurst); USUHS, Bethesda, MD (Letgers)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.39	2.14	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to obtain an increased understanding of hepatitis E virus (HEV) and related viruses with the goal of designing strategies for their control. Although sequence analysis of HEV clones is still rather limited, preliminary data indicate that this virus, like most single-stranded RNA-containing viruses, exhibits significant heterogeneity of genomic sequence. This has potential implications for vaccine development, since heterogeneity of the degree exhibited by this virus is sometimes associated with serotypic variation. However, the limited data available on cross-challenges in primate models suggest that even relatively divergent strains will elicit cross-protective antibodies.

1. A full-length cDNA clone of the Sar-55 strain of HEV has been constructed and is being tested for infectivity.
2. The major HEV structural protein has been obtained by expression of part of the Sar-55 genome in insect cells using a baculovirus expression vector.
3. An ELISA for anti-HEV IgG and IgM has been developed.
4. Prolonged infection of a small percentage of Vero cells in culture has been documented using the polymerase chain reaction.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-030

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00333-11 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Longitudinal and Cross-Sectional Studies of Viral Gastroenteritis in Infants & Young Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
	Judy F. Lew, M.D.	Sr. Research Investigator	LID, NIAID
	Mariam T. Wagner	Biologist	LID, NIAID

COOPERATING UNITS *(If any)*

Children's Hospital National Medical Center, Washington, DC (Kim)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.1	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses have been studied extensively in many parts of the world predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness in infants and young children. There has been a paucity of longitudinal viral gastroenteritis studies that yield not only important "denominator" data but also valuable insights into the natural history of a pathogen or illness, with special emphasis on epidemiologic, immunologic and laboratory parameters. We, therefore, initiated an intensive examination of anal swab and serum specimens obtained during a previous LID long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for homeless, but otherwise normal children. Our aim is to investigate the natural history of rotavirus infections in such a longitudinal setting employing recently developed techniques such as serotyping of rotaviruses with VP7 specific monoclonal antibodies, determining the epitope-specific serologic responses in sequential sera to determine the scope of homotypic and heterotypic responses in order to gain insight into the parameters influencing susceptibility or resistance to rotavirus disease. In addition with the availability of rotavirus strains obtained over 20 years ago, it is planned to compare such strains with current isolates at the genetic level to determine whether significant differences had occurred and the effect of such differences, if found, on resistance to illness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00339-11 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and Serotypic Characterization of Human and Animal Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Mitzi Sereno	Biologist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*

Ohio State University (Saif), Texas A & M University (Woode), Moredun Research Institute, Scotland (Snodgrass), University of Pavia, Italy (Gerna)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The bovine rotavirus B223 strain was determined to belong to VP7 serotype 10, the bovine rotavirus 678 strain was shown to belong to VP7 serotype 8, and the human rotavirus strains PA151 and PA169 were found to share neutralization specificity with bovine rotavirus VP7 serotype 6 strains. Five single VP7 gene substitution reassortants were generated using a human rotavirus strain ST3 (VP7 serotype 4) and bovine rotavirus strains UK, NCDV, B223, 678, or J2538, each of which had only the VP7 gene from the ST3 strain and the remaining 10 genes from the bovine strain. By cross-neutralization assay, the existence of at least four distinct bovine rotavirus VP4 serotypes was demonstrated. Single gene substitution reassortants were generated between porcine rotavirus strain Gottfried (VP7 serotype 4) and human rotavirus strains D (VP7 serotype 1), DS-1 (VP7 serotype 2), or M (VP7 serotype 3). These reassortants were each comprised of ten genes from the Gottfried strain and a single gene encoding VP7 from the human rotavirus strain. The three porcine x human reassortant viruses as well as parental Gottfried virus have successfully been adapted to grow in DBS-FRhL-2 diploid cells.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-654-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00340-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Studies of Rotavirus Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Mitzi M. Sereno	Biologist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
Ohio Agricultural and Development Center, Ohio State University, Wooster, OH (Saif)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continued efforts have been made to delineate the genetic mechanisms underlying virulence of rotavirus in the newborn gnotobiotic piglet model. A single gene substitution reassortant that had the 1st, 2nd, 5th, 6th, 7th, 8th, or 11th gene from human rotavirus DS-1 strain (which does not induce productive infection or diarrhea in gnotobiotic piglets) and the remaining ten genes from porcine rotavirus SB-1A strain (which consistently induces diarrhea in piglets) induced diarrhea in piglets that was associated with virus shedding. However, piglets challenged with a single gene substitution reassortant that had the VP3 gene, or VP4 gene, or VP7 gene, or NS-28 gene of DS-1 virus and the remaining ten genes from porcine SB-1A virus failed to develop illness or shed virus. Thus, we demonstrated that the 3rd, 4th, 9th (VP7), or 10th (NS28) gene each play an important role in virulence of rotavirus infection in piglets. Piglets challenged with a rotavirus reassortant that had the 3rd, 4th, 9th, and 10th genes of SB-1A virus and the remaining seven genes from DS-1 virus developed diarrhea and shed virus.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-554-030

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00341-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Live Attenuated Rotavirus Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(If any)*
Institute of Biomedicine, Caracas, Venezuela (Pérez-Schael); Univ. of Rochester (Madore, Christy); Johns Hopkins Univ. (Midthun); Univ. of Tampere (Vesikari, Ruuska); Institute of Nutrition, Peru (Lanata); Vanderbilt Univ. (Wright); Wyeth-Ayerst Research (Davidson); Secretech, Inc. (Schafer)

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SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.6	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses are the single most important etiologic agents of severe diarrhea in infants and young children in both developed and developing countries. It was estimated by the IOM that in the developing countries, annually, over eight million infants and young children develop severe rotavirus diarrhea, and over 870,000 die. Thus, there is an urgent need for a rotavirus vaccine that can prevent severe rotavirus diarrhea during the first two years of life when the disease is most serious. This is a major goal of Section. The "Jennerian" approach to vaccination, which involves the use of a live vaccine virus strain derived from a non-human host, has been evaluated in clinical trials using surrogate rotavirus strains of bovine origin (by others) or of simian rhesus monkey origin by us. This approach has had limited success because serotype-specific immunity against each of the four medically important human rotavirus VP7 serotypes could not be achieved consistently in infants less than six months of age with such a monovalent vaccine. We are currently pursuing a modified "Jennerian" approach in which a quadrivalent rotavirus vaccine containing (i) rhesus rotavirus (RRV) (VP7 serotype 3) and (ii) three human rotavirus-RRV reassortants, each possessing 10 RRV genes and a single human rotavirus gene that encodes VP7 serotype 1, 2, or 4 specificity, is given orally at two months of age. Placebo-controlled field trials with individual reassortant vaccines or the quadrivalent vaccine have been completed or are in progress. The vaccines appear to be quite promising, in general. A significant reduction in the incidence of rotavirus diarrhea in vaccinees when compared to placebo recipients has been observed in most, but not all, of the studies.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00342-11 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS *(if any)*

Epidemiology Division, US Naval Medical Research Institute, Rockville and Bethesda, MD, and Cairo, Egypt (Hyams, Bourgeois)

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Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The electron microscope is an indispensable component of a laboratory engaged in the study of gastroenteritis viruses. Two major groups of gastroenteritis viruses--the 27nm Norwalk virus and the 70nm human rotaviruses--were discovered at NIH and in Australia, respectively, (as well as the hepatitis A virus at NIH, in collaboration with the Hepatitis Section) by the use of electron microscope techniques. These agents were discovered without the use of an *in vitro* tissue culture system, because they could not be grown from clinical specimens in cell culture. The term, "direct virology," is apt to describe this method of examining viruses from clinical specimens by electron microscopy. Although second and third generation tests have been developed for the detection of the Norwalk group of viruses and the rotaviruses, the electron microscope is still an indispensable tool for the study of these gastroenteritis viruses. It is also the most rapid diagnostic method for detection of rotavirus from a clinical specimen and is the only method available for detecting infection with certain fastidious 27nm or similar small round virus-like particles from individuals with viruses associated with epidemic nonbacterial gastroenteritis. In addition, it is the only method that is capable of detecting all known gastroenteritis viruses (e.g., group A or non-group A rotavirus, Norwalk-like viruses, adenoviruses, astroviruses, caliciviruses) by examination of a single stool specimen. It has also been employed recently in collaborative studies for the visualization of baculovirus expressed Norwalk virus outer capsid antigen and a baculovirus expressed hepatitis E antigen.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00343-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Norwalk Virus and Related Norwalk-Like Viruses in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Judy Lew, M.D.	Sr. Research Invest.	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
Baylor College of Medicine (Estes); U.S. Naval Medical Research Institute (Hyams); University of Tampere, Finland (Vesikari)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	0.6	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The association of Norwalk virus and related 27nm viruses with acute, epidemic gastroenteritis is well established, but the full extent of the role of these viruses in enteric illness has been difficult to assess. Major advances in our understanding of the epidemiology of Norwalk virus were made this year using recombinant capsid protein from cloned Norwalk virus (provided to our laboratory by Dr. Estes, Baylor College of Medicine) as an antigen in an ELISA to detect serologic responses. With the availability of a relatively unlimited source of antigen, seroepidemiologic studies of Norwalk virus infection are now feasible. Preliminary serologic studies indicate that the Norwalk or a related virus is capable of infecting infants and young children more frequently than anticipated from prior limited studies. The association of such infections with illness is not known at present.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00346-10 LID

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Characterization of the Rotavirus VP4 Gene by Sequencing and Hybridization Techniques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	A. Duncan Steele, Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-809

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00446-08 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Testing of Rotavirus Vaccine Candidates in Venezuela and Peru

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
Instituto de Biomedicina, Caracas, Venezuela (Pérez-Schael); Instituto de Investigacion Nutricional, Lima, Peru (Lanata)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.7	0.8

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have conducted several phase I studies to evaluate the antigenicity and reactogenicity of various formulations of the rhesus rotavirus-based quadrivalent vaccine candidate in two month old infants. In these studies we aimed at achieving an arbitrary 50% "take rate" standard for each rotavirus serotype in the vaccine. In general, increase in the vaccine titer and the number of doses resulted in greater antigenicity. The quadrivalent vaccine was associated with mild, short-lived febrile episodes in about 25% of the infants tested. This level of reactogenicity was considered acceptable. A three dose schedule of the quadrivalent vaccine at a titer of 1 x 10^5 PFU of each component best approached the desired standard and is being evaluated in an efficacy trial in Venezuela.

We have also investigated the M37 rotavirus strain as an alternative vaccine candidate alone or in combination with RRV-based quadrivalent vaccine. M37 was safe but less immunogenic than the quadrivalent vaccine.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00451-04 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological and Molecular Characterization of Rotavirus Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid Section	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Kazuo Nishikawa, M.D.	Visiting Associate	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00507-06 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Rotavirus Serotypes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D	Sr. Staff Fellow	LID, NIAID

COOPERATING UNITS *(If any)*
University of Rochester, Rochester, NY (Madore & Dolin); University of Tampere, Tampere, Finland (Vesikari)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotavirus outer capsid proteins VP7 and VP4 independently induce neutralizing and protective antibodies in the host. Multiple VP7 and VP4 serotypes have been identified and different combinations of these two proteins have been found in circulating human rotavirus strains. Furthermore, antigenic variation within each protein has been described. The purpose of this project is to examine the molecular basis for serotypic differences among rotaviruses and to assess the role of antigenic diversity and variation in the epidemiology of the virus. This information is relevant to the development of rotavirus vaccine strategies.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-838

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00533-05 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Rotavirus Proteins with Monoclonal Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Green, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID, NIAID

COOPERATING UNITS *(if any)*
University of Tampere, Tampere, Finland (Vesikari)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major area of research in the Epidemiology Section is the development of a rotavirus vaccine. The first vaccine candidates to be evaluated in human efficacy trials by various groups were live, attenuated orally-administered bovine or simian rotavirus strains. Because these vaccines induced variable protection against rotavirus diarrhea in placebo-controlled phase II studies in young infants, this project was initiated with the intent of dissecting serotype-specific responses to the two major neutralization antigens following immunization and identifying laboratory correlates of resistance to rotavirus illness.

In the last four years, advances have been made by us and others in the molecular characterization of different rotavirus VP7 and VP4 serotype-specific antigenic sites. Three antigenic sites, A, B, and C were defined by an Australian research group on the outer capsid glycoprotein VP7 by sequence analysis of neutralization-resistant mutants selected in the presence of serotype-specific neutralizing monoclonal antibodies. Two major antigenic sites were identified on the outer capsid spike protein, VP4, by a similar approach. Serotype-specific monoclonal antibodies from the U.S., Australia, and Japan became available and several large surveys of the epidemiologic distribution of human rotavirus serotypes were conducted. It was observed that there are four predominant VP7 serotypes (1, 2, 3, and 4) worldwide that can circulate concurrently in the same geographic area. Our inability to predict the distribution of individual serotypes for a given season suggested that a rotavirus vaccine must simultaneously induce immunity against multiple circulating serotypes. By studying the repertoire of rotavirus-specific serum antibody responses in adults and infants who receive rotavirus vaccine candidates, we are attempting to delineate the immune correlates of protection against rotavirus illness.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-936

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00534-05 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Analysis of Non-Group A Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Kim Green, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS *(if any)*
Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio (Saif); Capital Institute of Pediatrics, Beijing, China (Qian)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although the major goal of the Epidemiology Section is the development of a vaccine against Group (Gp) A rotavirus, it is important to study other antigenically distinct groups of rotavirus (B-G) since viruses belonging to two of these groups, B and C, have been implicated as etiologic agents of diarrheal disease in humans. Group B rotaviruses have been associated with large outbreaks of severe diarrhea in adults. Curiously, these outbreaks have, thus far, remained limited to China. Sporadic outbreaks and isolated cases of diarrhea caused by Group C rotavirus have been reported in several countries and this group of agents appears to be associated with illness in an older population of children (school-aged) than that of group A rotaviruses (6 months to 2 years of age). It has been difficult to establish the epidemiology of the "non-group A" human rotaviruses because they cannot be cultivated in cell culture, making the development of reagents for diagnostic tests difficult. One objective of this project is to generate tools for epidemiologic studies. However, it is also of interest to examine the molecular basis for group differences among rotaviruses because it may give insight into the mechanisms that have caused Group A rotaviruses to predominate as an enteric pathogen.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00558-03 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prediction of Rotavirus Serotypes by Hybridization to Specific Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	A. Duncan Steele	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00573-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Rotavirus Proteins in *Salmonella* Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Green, Ph.D.	Sr. Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
University of Maryland, Baltimore, Maryland (Levine & Hone); Washington University (Curtiss); University of Missouri (Parker)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of mucosal immunity in the development of resistance to many of the pathogens that have been problematic for vaccine development, including rotaviruses, is poorly understood. This area of immunologic research is relevant to the development of vaccines against mucosal pathogens because there is evidence that the delivery of antigens to the site of viral or bacterial infection is important for achieving effective immunization. The goal of this project is to assess the feasibility of a recombinant rotavirus vaccine using *Salmonella* bacteria as an antigen delivery system. In addition, we will use this system to study mucosal immune responses to rotavirus antigens.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00574-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Distribution of Conserved and Serotype-Specific Epitopes on Rotavirus VP8

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
Universidad Central Venezuela (Larralde)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent studies have begun to elucidate the antigenic relationships among human rotaviruses on the basis of the antigenic specificity of the VP4 protein. A VP4 serotype system was recently established by the neutralization technique using guinea pig antiserum to the baculovirus recombinant-expressed VP4 protein of human rotavirus (HRV) strain KU, DS-1, 1076, or K8. The criterion for serotype specificity was a greater than eight-fold reciprocal difference in neutralizing antibody titer. Three distinct VP4 serotypes were defined, namely VP4 1, VP4 2 and VP4 3. In addition, two VP4 1 subtypes were identified, namely VP4 1A and VP4 1B.

In a recent study, the VP5 and VP8 cleavage subunits of VP4 from three different human rotavirus strains, KU (VP4 serotype 1A), DS-1 (VP4 serotype 1B), and 1076 (VP4 serotype 2), were independently expressed in *Escherichia coli*. Guinea pig antisera produced in response to immunization with these recombinant VP5 and VP8 proteins were used in cross-neutralization tests to determine the contribution of each of these two subunits to the overall antigenic specificity of VP4.

In addition, three peptides which collectively span the entire VP8 subunit of VP4 were also expressed in *E. coli*, and each peptide was analyzed by the immunoblot technique using different VP4 serotype antisera. Guinea pig antisera produced in response to immunization with these peptides were used in plaque reduction neutralization (PRN) assay to assess their specificity. The distribution of conserved as well as specific epitopes in the VP8 subunit of VP4 was studied.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00575-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of the Outer Capsid Protein of Human Rotaviruses in Infectivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gisela Larralde, M.S.	Guest Researcher	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1990-91

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00576-03 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Serotype Analysis and Characterization of Rotaviruses from Malaysia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Nassar Rasool, Ph.D.	Guest Researcher	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The distribution of rotavirus VP7 serotypes circulating in four locations in Malaysia, representing three geographical areas, was examined using 341 RNA-positive stool specimens obtained discontinuously between 1977-88 from hospitalized children below the age of five years suffering from acute gastroenteritis; the majority of specimens (70%) originated from 1988. A total of 306 specimens (256 stool suspensions and 50 tissue culture-adapted strains) were confirmatory ELISA-positive and were tested in a serotype ELISA utilizing monoclonal antibodies to rotavirus serotypes 1, 2, 3, 4, and 9. A total of 59% could be serotyped. Their serotype distribution demonstrated an overall predominance of rotavirus serotype 4 in Malaysia (71%), followed by serotype 1 (15%); serotypes 2 (4%) and 3 (4%) were rare. No serotype 9 rotavirus was detected, and eleven specimens (6%) displayed multiple serotype reactivities. Rotavirus serotype 4 predominated in 1988 in the three West Malaysian locations, while a more even serotype distribution was observed among a limited number of specimens from an East Malaysian location. Serotype 4 also predominated in Kuala Lumpur in 1987; limited data suggested the possible predominance of serotype 1 in this location in earlier years. All 341 specimens were also passaged twice in AGMK cells for tissue culture adaptation, followed by one passage in MA-104 cells. The VP4 types of all specimens that were successfully adapted to growth in MA-104 cells was determined by molecular hybridization of northern-blotted RNA to PCR generated probes representing the hyperdivergent region of the VP8 portion of the VP4 genes of rotavirus strains KU, DS-1, 1076, and K8 (VP4 serotypes 1A, 1B, 2, and 3, respectively). The majority (123) of specimens tested hybridized with the KU probe, three specimens hybridized specifically with the K8 probe, and none hybridized to the 1076 probe.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00582-02 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression and Recovery of Recombinant Rotavirus VP4 from Insect Cell Cultures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Luis G. Juarbe-Osorio	Guest Researcher	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00583-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of the Outer Capsid Protein VP4 of Human and Porcine Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Baoguang Li, Ph.D.	Visiting Associate	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-030

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00601-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Recombinant BCG-VP8 Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Mitzi Sereno	Biologist	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
MedImmune Laboratory (Stover)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses are major etiologic agents of severe diarrheal disease in infants and young children throughout the world. Neutralizing antibodies to rotaviruses have been shown to be induced independently by two outer capsid proteins, VP7 and VP4; these antibodies are associated with resistance to illness.

The baculovirus expression system has been used to successfully express the VP4 of various rotavirus strains. The protein synthesized in this system exhibits native conformation based on its reactivity with polyclonal and available monoclonal antibodies (MAbs). In addition, guinea pigs immunized with such proteins developed antibodies that neutralized infectivity of the immunizing rotavirus efficiently. Moreover, immunization of guinea pigs with the expressed VP8 or VP5 cleavage subunit of VP4 induced antibodies that also neutralized rotavirus infectivity. Results obtained by cross-immunoprecipitation and by neutralization assay with these antisera suggest that the VP8 subunit of VP4 contains the major antigenic sites responsible for the neutralization of rotavirus via VP4.

A major objective of this study was to characterize the VP4 protein of the murine rotavirus Eb strain (VP7 serotype 3) and define the mucosal immune response to this outer capsid protein in the homologous mouse model. We chose as a mucosal vector the human tuberculosis vaccine, bacille Calmette-Guerin (BCG), an attenuated strain of mycobacterium bovis that has been used extensively and safely in humans. BCG vaccine is commonly given at birth and elicits a long-lasting immune response with a single dose. It is also a highly effective adjuvant.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00602-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Rotavirus Outer Capsid Protein VP4 by Recombinant-Adenovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
Wyeth-Ayerst Research Laboratories (Davis & Selling)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses are the major cause of severe diarrhea in infants and young children in both developed and developing countries. Therefore, intensive efforts are being made to develop an effective vaccine. Two outer capsid rotavirus proteins, VP7 or VP4, are each associated with the induction of neutralizing antibodies that have been shown in experimental animals to be associated with resistance to illness. Serotype specificity has been associated with VP7. However, recent studies have begun to elucidate the antigenic relationships among human rotaviruses based on the antigenic specificity of the VP4 protein as well. Human rotavirus strains that were associated with symptomatic infection and that exhibited VP7 specificity of serotype 1, 2, 3, 4, or 9 each possessed a similar VP4 as determined by neutralization assay.

We have chosen the outer capsid protein VP4 of porcine rotavirus OSU as a model to study the immunogenicity of this protein in an adenovirus vector. The ability of adenovirus to be administered orally makes the potential use of adenovirus vectors for vaccine production particularly attractive. In addition, adenovirus vaccines have already been shown to be safe in humans when administered orally as enteric coated capsules or tablets.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00603-02 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monoclonal Antibodies Directed to VP8 Subunit of VP4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Ferdinando Liprandi	Guest Researcher	IVIC, Venezuela

COOPERATING UNITS *(If any)*

UCV, Venezuela (Larralde)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously, antisera against recombinant outer capsid rotavirus VP4 proteins were used to study the VP4 antigenic polymorphism among human rotavirus strains. Three distinct serotypes and one subtype of the VP4 protein were identified among 17 human rotavirus strains that contained representatives of five different VP7 serotypes. Results from cross-immunoprecipitation and neutralization assays with VP8 antisera suggest that the VP8 cleavage subunit of VP4 contains the major antigenic sites responsible for the serotype-specific neutralization of rotavirus via VP4.

Immunization of mice with the VP8 cleavage subunit of VP4 of the human rotavirus KU, DS-1, 1076, or K8 strain induced polyclonal antibodies that neutralized the rotavirus from which the VP4 subunit was derived. However, most of the VP4-monoclonal antibodies obtained so far have recognized heterologous strains. We will attempt to produce serotype-specific monoclonal antibodies (MAbs) against VP4 using "mosaic virus" produced by coinfection of insect cells with baculovirus recombinant expressing VP6, VP7, and VP4 genes.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00604-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cold-Adaptation of Human Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
National Institutes of Health, Tokyo, Japan (Matsuno)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two human rotavirus cell-culture isolates (strains D [VP4:1A; VP7:1], and DS-1 [VP4:1B; VP7:2]) as well as two human x human rotavirus reassortants (Wa x P [VP4:1A; VP7:3] and Wa x DS-1 [VP4:1A; VP7:2]) have been successfully adapted to grow in primary African Green Monkey kidney cells at the suboptimal temperature of 30°C, 28°C, or 26°C. These rotavirus strains were cold-adapted to determine whether cold-adapted (*ca*) mutants selected by this strategy are attenuated and suitable for use in a live virus vaccine. The 26°C and 28°C *ca* mutants of the D, DS-1, Wa x P, and Wa x DS-1 viruses have been plaque-purified and their phenotypes have been characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00605-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Infection of Chimpanzees by Human Rotavirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
Georgetown University/Twinbrook (London); Bioqual, Inc., Rockville, MD (Bradbury)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<0.1	<0.1	<0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our current rotavirus vaccination strategy involves the use of an animal rotavirus strain of simian origin in combination with three human rotavirus-rhesus rotavirus reassortant strains to form a quadrivalent vaccine in an attempt to achieve protection against each of the four medically important VP7 rotavirus serotypes. If this approach which relies on the induction of antibody to rotavirus outer capsid protein VP7 fails to achieve the desired level of protective efficacy, we are considering an alternative strategy: the use of one or more cold-adapted human rotavirus mutants. These mutants would possess the human rotavirus VP4 (whereas the quadrivalent vaccine possesses the rhesus rotavirus VP4), an important outer capsid protein that may be important in the induction of heterotypic immunity. It is important to develop an animal model that develops diarrhea following oral administration of a virulent human rotavirus strain in order to determine if the cold-adapted human rotavirus mutant is attenuated. We administered a virulent VP7 serotype 1 human rotavirus strain to two susceptible chimpanzees by the alimentary route. One chimpanzee developed loose stools four to six days after inoculation, shed rotavirus on day 2 to day 7, and developed a seroresponse to the serotype 1 virus. The other chimpanzee developed loose stools beginning on the third or fourth day that subsided before the fifth day after inoculation. The latter animal did not shed rotavirus and failed to develop a seroresponse to serotype 1 rotavirus by PRN assay.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-830

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00606-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Neonatal Rotavirus Strains Detected in South Africa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Duncan Steele, Ph.D.	Visiting Fellow	LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00639-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Epidemiology of Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
Medical University of Southern Africa (Steele); Institute of Virology, University of Pavia, Pavia, Italy (Gerna)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 0.9	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular epidemiology of human and animal rotaviruses is being studied by hybridization and sequencing techniques with four major objectives: (1) investigate the role of outer capsid protein VP4 in the virulence of human rotaviruses; (2) develop hybridization probes specific for the genes of VP4 and VP7 (the two outer capsid proteins) for identification of rotavirus serotypes and delineation of their relative distribution; (3) identify and characterize new VP4 and VP7 serotypes in human and animal rotavirus strains; and (4) determine the relative proportion of the neutralizing antibody response to natural rotavirus infection that is specific for each of the outer capsid proteins, VP4 and VP7.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00640-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthetic Analogs of Rotavirus Genomic RNA Expressing a Foreign Marker Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
	An-Dao Yang, M.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses are the most important cause of viral gastroenteritis in humans and animals. The rotavirus genome consists of 11 segments of double-stranded (ds) RNA of 667 (segment 11) to 3,302 bp (segment 1). The general features of rotavirus transcription and replication probably are similar to those described for reovirus. Virions possess a viral polymerase which is contained together with the dsRNA segments in a subviral single-shelled core and directs copying of the parental minus strand into progeny plus strands (transcription). The plus strands serve as mRNAs and also as templates for the synthesis of progeny minus strands to yield dsRNA (replication). During the synthesis of the negative strand the nascent dsRNA associates with viral proteins to form subviral cores which eventually are incorporated into virions.

The 5' and 3' ends of rotavirus RNAs contain distinct, unrelated terminal consensus sequences of 7 to 10nt which are assumed to be important *cis*-acting signals. These presumably include, at the 3' ends, the viral promoters for the synthesis of plus and minus strands. The termini also might contain sequences important for packaging and for regulation of expression at the levels of transcription, replication and translation. The 5' end of the plus strand has a methylated cap structure and neither strand is polyadenylated.

Recently, it was shown by others that authentic plus-sense reovirus RNAs synthesized *in vitro* by subviral cores were infectious when transfected into cells and complemented with a helper reovirus from a different serotype. To date, however, similar results have not been reported with synthetic reovirus RNAs encoded by cDNA or with any rotavirus. The capability of introducing synthetic RNAs into infectious virus would provide the basis for performing detailed structure-function studies of the viral RNAs and proteins and for developing methods for engineering attenuated vaccine strains. Our approach has been to develop a simplified system in which cDNA-encoded synthetic analogs of a rotavirus RNA, consisting of the bacterial chloramphenicol acetyl transferase (CAT) reporter gene flanked by the 5' and 3' gene 9 noncoding regions, were rendered biologically active by transfection into rotavirus-infected cells. This provides a new, sensitive system for characterizing rotavirus gene replication and expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00641-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Neutralizing Epitopes on Rotavirus Outer Capsid Proteins (VP4 and VP7)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two monoclonal antibodies which exhibited neutralizing activity against the outer capsid VP7 of two or more rotavirus serotypes were utilized to locate amino acid residues invlved in the formation of cross-reactive epitopes. Neutralizing monoclonal antibody (N-mAb) raised against the VP7 of porcine rotavirus Gottfried strain (VP7 serotype 4) neutralizes not only VP7 serotype 4 strains but also VP7 serotype 3, 6, 9, or 10 viruses. N-mAb raised against the VP7 of rhesus monkey rotavirus MMU 18006 strain (VP7 serotype 3) neutralizes serotype 3 viruses as well as porcine serotype 4 viruses. Neutralization-resistant variants were selected in the presence of these monoclonal antibodies.

Analysis of nucleotide and amino acid (aa) changes in antigenic variants of serotype 3, 4, 6, 9, or 10 rotavirus selected in the presence of the serotype cross-reactive N-mAbs showed that: (1) in addition to variable regions VR-5 (aa 87-100) and VR-7 (aa 141-150), variable regions VR-8 (aa 208-224) and VR-9 (aa 235-242) are involved in cross-reactive neutralization; (2) the site of amino acid substitution on mutant VP7 selected by a single N-mAb can vary, resulting in mutants which exhibit antigenic differences; (3) an amino acid substitution can occur at the same position on the VP7 of different serotypes selected by a single N-mAb; or (4) the location of an amino acid substitution on the mutant VP7 selected by a single N-mAb can vary depending on the rotavirus serotype.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00642-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Norwalk and Norwalk-like Viruses (27nm viruses)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Judy F. Lew, M.D.	Sr. Research Investigator	LID, NIAID
Others:	Kim Y. Green, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(If any)*
Baylor College of Medicine, Houston, Texas (Estes)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although Norwalk virus and Norwalk-like agents have been shown to be the most common cause of epidemic nonbacterial gastroenteritis, at present almost nothing is known about the molecular epidemiology of these 27 nm viruses. Our goal is to determine what molecular characteristics are important in the epidemiology, immunogenicity, and pathogenesis of these agents. Thus far, we have cloned and sequenced part of the polymerase region of several 27 nm viruses from the Middle East and Canada to compare with that of the Norwalk virus which has just recently been sequenced. Also, we have now amplified the capsid region of the Norwalk virus by PCR and plan to do the same for other 27 nm viruses in order to generate clones for use in future protein expression studies.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00643-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Rotavirus Outer Capsid Protein VP4 by a Baculovirus Recombinant

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Scientist	LID, NIAID
Others:	Baoguang Li, Ph.D.	Guest Researcher	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses are ubiquitous agents that cause diarrheal disease in the young of not only humans but also many animal species. Several candidate vaccines against human rotavirus (HRV) disease are currently being evaluated with special emphasis on the VP7 outer capsid protein, which is a major protective antigen. Currently, eight different serotypes have been established among HRVs based on the antigenic specificity of VP7. The outer capsid protein, VP4, is also a major protective antigen, but relatively little is known about the antigenic relationships and extent of polymorphism of the VP4 of various HRVs. In previous studies, it was shown by sequence analysis that at least four alleles of the VP4 gene are present among HRVs.

One allele of VP4 is present on strains recovered from patients with symptomatic infection. These strains had been classified as VP7 serotype 1 (strains KU and Wa), VP7 serotype 3 (strain P), or VP7 serotype 4 (strain VA70). These rotavirus strains, designated as VP4 genetic group 1, possess a highly conserved VP4 protein that exhibits 93-97% amino acid identity. A second allele of VP4 is present in HRVs belonging to VP7 serotype 2 (strains DS-1 and RV-5) that exhibit 98% VP4 amino acid identity. These strains, designated as VP4 genetic group 2, were also recovered from symptomatic infections. Moreover, the VP4s of genetic group 2 are most closely related to the corresponding proteins of group 1, exhibiting an 89-90% amino acid homology. A third allele of VP4 is present in HRV strains that were recovered from asymptomatic neonatal infections and that belong to VP7 serotype 1 (M37), VP7 serotype 2 (1076), VP7 serotype 3 (McN), or VP7 serotype 4 (ST3). The VP4s of these strains (designated VP4 genetic group 3) are highly related, as indicated by 95-97% amino acid identity. The VP4s of the third genetic group exhibit only a 75% amino acid identity with the corresponding VP4 proteins of groups 1 and 2. A fourth allele is present on the HRV K8 strain (belonging to VP7 serotype 1) that appears to have a unique VP4 gene exhibiting only 64-65% amino acid identity with the VP4 proteins of viruses of VP4 genetic group 1, 2, or 3.

In a recent study, four different VP4 alleles present on HRV strains KU (genetic group 1), DS-1 (genetic group 2), 1076 (genetic group 3), and K8 (genetic group 4) were separately expressed in *Spodoptera frugiperda* (Sf9) cells using the baculovirus vector *Autographa californica* nuclear polyhedrosis virus. Antisera produced in response to immunization with these recombinant VP4 proteins allowed us to define the extent and distribution of VP4 antigenic polymorphism among rotaviruses currently circulating in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00323-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Parainfluenza Virus Type 3 (PIV3) Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Microbiologist	LID, NIAID
Others:	Kenneth D. Dimock, Ph.D.	Special Volunteer	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.2	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new, simple system was developed for the direct manipulation of sequences of the genomic RNA (vRNA) of human parainfluenza virus type 3 (PIV3). Specifically, cDNA was constructed to encode a truncated synthetic vRNA analog in which all of the viral genes have been removed and replaced by the bacterial chloramphenicol acetyl transferase (CAT) gene. The cDNA is under the control of the promoter for T7 RNA polymerase and, at the distal end, contains an *HgaI* site for linearization. Transcription of the cDNA *in vitro* yields a negative-sense RNA containing the exact, correct vRNA termini. The RNA was transfected into tissue culture cells which were infected with PIV3 to supply complementing proteins. Under these conditions, the RNA was rendered biologically active ("rescued") as evidenced by the intracellular expression of CAT and by the production of a component (presumably infectious particles) that was released into the medium and could be passed productively onto fresh cells in the presence of helper virus. Together with a similar system developed by us for human respiratory syncytial virus (RSV) (accompanying report) and by others for Sendai virus, this represents the first experimental method by which one can synthesize, and thereby manipulate, biologically-active analogs of the vRNA of a nonsegmented negative strand virus. It will now be possible to directly identify and characterize *cis*-acting signals in vRNA. It should also be possible to modify the system such that rescue is complemented by cDNA-encoded viral proteins in place of PIV3. This would make it possible to characterize the roles of the proteins in replication, transcription and virion morphogensis. Importantly, the success in rescuing PIV3-CAT supports the idea that it ultimately will be possible to rescue a complete, nondefective synthetic vRNA encoding infectious virus.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-030

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00324-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Influenza Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Kanta Subbarao, M.D.	Senior Staff Fellow	LID, NIAID
	Cassandra Lawson, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*
University of Rochester (Treanor); Mt. Sinai School of Medicine (Palese); St. Jude Children's Hospital (Webster)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.525	1.925	1.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The single-gene reassortant (SGR) virus possessing a PB2 gene from an avian influenza A virus and the remaining genes from a human influenza A virus failed to produce plaques on mammalian cell monolayer culture and was restricted in replication in monkeys. Revertant viruses, which replicated and produced plaques efficiently on mammalian cells, possessed an amino acid substitution at residue 627 (glutamine in the avian PB2 gene → lysine in the revertant) and replicated to moderate levels in monkeys. The amino acid at position 627 in every avian virus PB2 gene sequenced to date is glutamic acid and in every human virus gene it is lysine. Thus the amino acid at position 627 on the PB2 protein is an important determinant of host range of influenza virus as well as virulence of these viruses for primates.

Gull-human influenza A virus reassortants derived from three different gull influenza A viruses were attenuated for squirrel monkeys or chimpanzees and, therefore, are candidate live attenuated viruses for use as vaccines in humans.

Ten distinct amino acid substitutions in the human influenza A virus PB2 protein that each separately specify the temperature-sensitive phenotype were identified. Previous studies had demonstrated that influenza A viruses with *ts* mutations were attenuated in humans. Techniques are being developed to introduce one or more of these *ts* mutations into a synthetic copy of the PB2 gene and to rescue this synthetic gene into an infectious virus.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00325-11 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Respiratory Viruses in Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Susan L. Hall, Ph.D.	Senior Staff Fellow	LID, NIAID
	James Crowe, M.D.	Research Associate (CO)	LID, NIAID
	Peter L. Collins, Ph.D.	Microbiologist	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	E. Kanta Subbarao, M.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

Bioqual Inc., Rockville (Bradbury); Georgetown University (London); St. Louis University (Belshe)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.275	0.925	1.35

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human parainfluenza virus type 3 (PIV3) cold passaged (cp) vaccine, cp45, was found to be highly attenuated for both the upper and lower respiratory tract of the chimpanzee. Infection of chimpanzees with the cp45 virus induced a high level of resistance to subsequent PIV3 wild type virus challenge. The attenuation and *ts* phenotypes of the cp45 virus were stable following replication in seronegative animals. These results are encouraging and provide a basis for proceeding to clinical trials in seronegative human infants and children.

The evaluation of three previously developed live attenuated RSV candidate vaccine strains was initiated. These three RSV temperature-sensitive (*ts*) mutants were characterized extensively in chimpanzees. The three mutants, RSV A2 *ts*-1, *ts*-1 NG-1, and *ts*-4 were similar in their level of attenuation and stability of the *ts* phenotype. Each mutant was effective in inducing resistance to subsequent RSV challenge. However, these viruses retained sufficient virulence for the upper respiratory tract to preclude their use as vaccines. A strategy for producing and identifying more satisfactory candidate RSV vaccines was devised. These vaccine candidates will be identified by comparing their effects in chimpanzees with those previously observed for the *ts*-1 mutants; specifically, their level of attenuation and genetic stability in seronegative chimpanzees. Those candidates more attenuated and more stable than *ts*-1 will be selected for subsequent studies in humans.

Gull-human influenza A reassortant viruses were attenuated and immunogenic in non-human primates and show promise for use as vaccines in humans.

U.S GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00326-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Respiratory Viruses in Volunteers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	E. Kanta Subbarao, M.D.	Senior Staff Fellow	LID, NIAID
	Susan Hall, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
PRI Dyn Corp., Rockville, MD (Potash); Johns Hopkins University, Baltimore, MD (Clements); St. Louis University, St. Louis, MO (Belshe)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.45	0.75

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A live attenuated bovine parainfluenza type 3 virus was infectious, non-reactogenic, and restricted in replication in eight human seronegative humans. It induced a serum antibody response against the human PIV3 and thus continues to show promise as a vaccine candidate.

The cold passaged mutant (cp45) of the JS wild type PIV3 was previously found to be safe in adults, seropositive children, and seronegative chimpanzees. Studies were initiated in seronegative infants and young children at a dose of $10^{4.0}$pfu and the very limited data that has been obtained to date indicates that the virus is attenuated and infectious.

Influenza A virus heterosubtypic immunity (*i.e.*, immunity induced by infection with one subtype of influenza A virus that is active against a virus belonging to another subtype) did not appear to be sufficient to affect the infectivity, immunogenicity, or replication of an attenuated influenza A virus vaccine in infants and young children. These observations confirm the weak nature of heterosubtypic immunity in humans, and suggest that it will not limit the utility of live attenuated influenza A viruses in young children.

Studies with live attenuated cold-adapted influenza A virus vaccine in infants less than six months of age were initiated. A dose of $10^{6.0}$TCID$_{50}$ was safe in this age group and about 40% of vaccinees could be reinfected with vaccine within two months of vaccination.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00327-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Parainfluenza Type 3 Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	Susan L. Hall, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Anne Stokes, Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 2.9	PROFESSIONAL: 1.9	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The JS strain of PIV3 is the wild type parent of three candidate live attenuated cold-passaged (*cp*) viruses; namely cp12, cp18, and cp45. In order to develop an understanding of the genetic basis of attenuation of the mutant viruses, the nucleotide and deduced amino acid sequence of the JS *wt* and cp12 and cp45 mutant viruses was undertaken. The complete sequence of the JS wild type PIV3 has been obtained and that of the cp12 and cp45 mutants is almost completed. The most promising attenuated vaccine candidate, the cp45 virus, contains at least 16 nucleotide changes from the JS wild type virus (a few nucleotide differences remain to be confirmed). We are using the cDNA clones employed to sequence the JS wild type and cp45 viruses to construct complete cDNA copies of each of these two viruses, a project that is 70% complete. A strategy is being developed to produce an infectious virus from RNA transcribed from the complete copy of PIV3 which will greatly accelarate our ability to produce live attenuated PIV3 vaccines.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00345-11 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Peter Collins, Ph.D.	Microbiologist	LID, NIAID
	Mark Connors, M.D.	Research Associate (CO)	LID, NIAID
	James Crowe, M.D.	Research Associate (CO)	LID, NIAID
	Arun Kulkarni, Ph.D.	Visiting Fellow	LID, NIAID

COOPERATING UNITS *(if any)*
Upjohn Company, Kalamazoo, MI (Wathen); LIP/NIAID/NIH (Morse); Wyeth-Ayerst Research, Radnor, PA (Davis); Scripp Research Institute (Burton); PRI, La Jolla, CA (Zebedee)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.65	2.85	1.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human monoclonal antibodies to respiratory syncytial virus (RSV) are being developed for passive immunoprophylaxis of infants and children who are at high risk for serious lower respiratory tract disease due to RSV as well as for therapy of serious RSV disease. Two genetically distinct Fab fragments of IgG were selected from a combinatorial library expressed on the surface of filamentous DNA bacteriophage. These Fabs are specific for the F glycoprotein of RSV and have high neutralizing activity. These observations suggest that it should be possible to develop whole (*i.e.*, Fab + Fc) IgG antibodies that will be useful for prevention and therapy of serious RSV lower respiratory tract disease.

The vaccinia-RSV recombinant expressing the RSV M2 gene induced resistance in mice that was mediated by $CD8^+$ T-cells. The level of primary pulmonary $CD8^+$ cytotoxic T-cell (CTL) activity correlates with the resistance induced. The resistance induced by Vac-M2 (but not that induced by Vac-F or Vac-G) and the pulmonary CTL activity were short-lived and were not demonstrable 45 days following immunization. This suggests that inclusion of CTL epitopes in vaccines may not be important for vaccine efficacy since resistance to reinfection is primarily mediated by antibodies.

Mice immunized with formalin-inactivated RSV (FI-RSV) vaccine did not develop RSV-specific CTLs, whereas mice infected with RSV developed high levels of CTL activity. This finding, considered in the context of last year's observation that FI-RSV vaccine preferentially stimulates CD4 cells, supports the view that a primary defect of the inactivated RSV vaccine was an imbalance in the T-cell response. This unbalanced cell-mediated immune response likely played a major role in disease potentiation that occurred in the 1960s when young FI-RSV vaccinees underwent subsequent RSV infection.

A program to develop live attenuated RSV vaccines was initiated and several mutants have been identified that could be useful as RSV vaccines.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-654-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00368-10 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Analysis of the Genome of Respiratory Syncytial Virus (RSV)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Microbiologist	LID, NIAID
Others:	David S. Stec, Ph.D.	NRC Fellow	LID, NIAID
	Haim Grosfeld, Ph.D.	Visiting Scientist	LID, NIAID
	Michael A. Mink, Ph.D.	IRTA Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been exploring methods of obtaining biological activity for cDNA-encoded synthetic genomic RNA (vRNA) of human respiratory syncytial virus (RSV). The ultimate goal is to produce infectious virus from a complete synthetic vRNA. This capability would provide an experimental system for analyzing the functions of RSV nucleic acid signals and proteins, and also would be invaluable for the production and characterization of engineered attenuated vaccine viruses. We have constructed cDNAs encoding vRNA analogs in which all of the viral genes have been deleted and replaced with the bacterial chloramphenicol acetyl transferase (CAT) marker gene. When transfected into tissue culture cells and complemented by infection with RSV, these truncated, negative-sense vRNA analogs were "rescued" in that they were expressed, amplified and packaged into infectious particles. Substantial progress has been made in identifying and characterizing *cis*-acting nucleotide signals important in rescue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00372-10 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Roles of RSV Proteins in Host Immunity and Molecular Approaches to Vaccine Design

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Microbiologist	LID, NIAID
Others:	Haim Grosfeld, Ph.D.	Visiting Scientist	LID, NIAID
	Mark Connors, M.D.	Research Associate (CO)	LID, NIAID
	James Crowe, M.D.	Research Associate (CO)	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Brian R. Murphy, M.D.	Head, RVS	LID, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.75	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are investigating approaches for utilizing recombinant DNA techniques for developing vaccines to human respiratory syncytial virus (RSV). Previously, recombinant vaccinia viruses or adenoviruses were constructed that express individual RSV proteins including the major protective antigens, the F and G glycoproteins. Efficacy tests in rodents and monkeys provided highly encouraging results, but in chimpanzees the recombinants proved to be insufficiently immunogenic and protective. Improvements in vector technology will be required before this approach will be feasible for RSV immunoprophylaxis. Construction of a series of adenovirus vectors containing alternative engineered insertion sites did not yield improved levels of expression or immunogenicity in rodents.

A second approach involves efforts to develop methods for producing infectious RSV from cDNA. This would make it possible to produce and characterize attenuated strains by direct genetic engineering. To date, we have successfully "rescued" a cDNA-encoded vRNA analog which is 49.3% of full-length and which contains an enzyme marker gene under the control of RSV transcriptive signals. Standard infectious RSV was used as helper to provide proteins to complement the cDNA-encoded vRNA analog. This analog was packaged into infectious particles and was passed five times in culture with undiminished efficiency. This provides support for the idea that a complete, nondefective cDNA-encoded vRNA can be rendered biologically-active to yield infectious virus.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00498-06 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis, Processing and Functions of the Proteins of Human RSV

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Microbiologist	LID, NIAID
Others:	Haim Grosfeld, Ph.D.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.4	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We would like to have a detailed picture of the processing, functions and assembly of the proteins of human respiratory syncytial virus (RSV). In previous years, we determined the primary structures of the viral proteins from sequencing cloned viral genes. This also identified various protein domains that were either tolerant or intolerant of amino acid substitutions, and also provided other clues to protein function. The folding, processing, and oligomerization of the F and G transmembrane glycoproteins were studied in detail using biochemical and cDNA mutation/expression techniques. This is now being extended to the third transmembrane glycoprotein, the SH protein. Also, we recently developed an experimental system in which *in vitro*-synthesized, truncated analogs of viral genomic RNA are transfected into tissue culture cells and rendered biologically active by complementation with viral proteins supplied by RSV superinfection. We are now working to achieve complementation using cDNA-expressed viral proteins in place of RSV. This would allow us to identify the viral proteins required for transcription, replication and virus production and would subsequently allow us to perform detailed structure-function studies of individual proteins.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-938

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00476-06 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Engineering the Genome of Dengue Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Hiroyuki Hori, M.D.	Visiting Associate	LID, NIAID
	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-030

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00477-06 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Analysis of Dengue Virus Envelope Glycoprotein and its Immunogenicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ruhe Men, M.D.	Special Volunteer	LID, NIAID
Others:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*
WRAIR, Washington, DC (Eckels)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00500-06 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Processing and Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID
	Lewis Markoff, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous results from our laboratory established that the N-terminal 70% of NS2A and the C-terminal 8 amino acids of NS1 are required for NS1-NS2A cleavage. To further delineate the requirements for NS1-NS2A cleavage, we constructed recombinant vaccinia viruses expressing chimeric proteins consisting of PreM fused to the 3 or 8 C-terminal amino acids of NS1 plus NS2A. The results showed that all of NS1, with the exception of the 8 amino acid cleavage site, is dispensable for NS1-NS2A cleavage. Also, a signal sequence is required for cleavage to occur as the chimeric protein Sig$^-$PreM-NS1(8)-NS2A was not cleaved. This suggests that targeting to the exocytic pathway is a prerequisite for NS1-NS2A cleavage. Additional experiments were performed to analyze this cleavage. Brefeldin A, a drug which blocks transport out of the Golgi, did not block NS1/NS2A cleavage. This suggests that NS1/NS2A cleavage occurs in the Golgi or in a compartment between the ER and the Golgi. NS1/NS2A cleavage was studied during a chase under conditions that block ER-to-Golgi transport: incubation with the drug carbonyl cyanide m-chlorophenylhydrazone (CCCP), or incubation at 14°C. Both of these treatments completely blocked the chase of uncleaved NS1-NS2A precursor into NS1 product. This is consistent with the notion that NS1/NS2A cleavage occurs after exit from the ER. Further elucidation of the cleavage mechanism is now possible using an *in vitro* ER-to-Golgi transport system recently developed by others.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-938

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00501-05 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of the Processing of Dengue Viral Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00502-05 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigenic Analysis of the Dengue Virus Envelope Glycoprotein (E) Using Synthetic Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
FDA (Berkower); Torrey Pines Institute for Molecular Studies, La Jolla, CA (Houghton)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00531-05 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Analysis of Dengue Nonstructural Proteins NS2B and NS3

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVBS	LID, NIAID
Others:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously showed that the NS3 protease domain is required for NS2A/NS2B and NS2B/NS3 cleavage. In addition, we showed that NS2B is also required for NS2A/NS2B, NS2B/NS3 and NS3/NS4A cleavage, and that NS2B can function in *trans*. DEN4 NS2B contains 130 amino acids, most of which are hydrophobic. We have proposed that NS2B plus NS3 interact to form a two component protease. Using polymerase chain reaction (PCR), we introduced seven new unique restriction enzyme sites into NS2B (5'-Nhe I, Stu I, Afl II, Apa I, Nru I, Spe I, Bst EII-3'). These new sites did not change the amino acid sequence of NS2B. In addition to the seven unique sites introduced, there is a unique Bgl II site in NS2B, located between the Apa I and Nru I sites. Oligonucleotides were used to bridge between the nearest-neighbor pairs of all 8 sites, creating seven internal deletion mutants of NS2B. Additional recombinants designed to further define the critical residues of NS2B have also been constructed. Analysis of this series of deletion mutants showed that a 40 amino acid domain is sufficient to mediate cleavage of the resulting NS2B-NS3 polyprotein. The critical NS2B sequence contains several charged amino acid residues, presumably important for interactions with the NS3 protease components.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00556-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epitopes on Dengue Virus Nonstructural Protein NS1 that Mediate Protection Against Dengue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hiroyuki Hori, M.D.	Visiting Associate	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*
Rochester General Hospital, University of Rochester, Rochester, NY (Schlesinger)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-1992

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00557-03 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cross-Reactive Antibodies to Clotting Factors in Patients Infected with Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID

COOPERATING UNITS *(if any)*

WRAIR, Bangkok, Thailand (Innis)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-564-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00571-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Amino Acid Substitution at the NS1-NS2A Cleavage Junction of Dengue Virus Polyprotein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michèle Pethel	Microbiologist	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously shown that proper processing of dengue type 4 virus (DEN 4) NS1 from the NS1-NS2A region of the viral polyprotein requires a hydrophobic N-terminal signal and the downstream NS2A. Results from deletion analysis indicate that a minimum length of eight amino acids at the C-terminus of NS1 is required for cleavage at the NS1-NS2A junction. Comparison of this eight amino acid sequence with the corresponding sequences of other flaviviruses suggests a consensus cleavage sequence of Met/Leu-Val-Xaa-Ser-Xaa-Val-Xaa-Ala. Site-directed mutagenesis was performed to construct mutants of NS1-NS2A that contained a single amino acid substitution at different positions of the consensus cleavage sequence or at the immediate downstream position. Three to eight different substitutions were made at each position. A total of 50 NS1-NS2A mutants were analyzed for their cleavage efficiency relative to that of the wild type DEN 4 sequence. As predicted, nearly all substitutions at positions P-1, P-3, P-5, P-7, and P-8, occupied by conserved amino acids, yielded low levels of cleavage, with the exception that Pro or Ala substituting for Ser (P-5) was tolerated. Substitutions of an amino acid at the remaining positions occupied by nonconserved amino acids generally yielded high levels of cleavage. However, some substitutions at nonconserved positions were not tolerated. For example, substitution of Gly or Glu for Gln (P-4) and substitution of Val or Glu for Lys (P-6) each yielded a low level of cleavage. Overall, these data support the proposed cleavage sequence motif deduced by comparison of sequences among the flaviviruses. In addition to the eight amino acid sequence, this study also showed that the amino acid immediately following the NS1-NS2A cleavage site plays a role in cleavage.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00572-03 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Processing of Dengue Virus Polyprotein NS3-NS4A-NS4B-NS5

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MBV Section	LID, NIAID
Others:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID
	Annie Cahour, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cleavage mechanism utilized for processing of the NS3-NS4A-NS4B-NS5 domain of the dengue virus polyprotein was studied by using the vaccinia virus expression system. Recombinant vaccinia viruses vNS2B-NS3-NS4A-NS4B-NS5, vNS3-NS4A-NS4B-NS5, vNS4A-NS4B-NS5, and vNS4B-NS5 were constructed. These recombinants were used to infect cells and the labelled lysates were analyzed by immunoprecipitation. Recombinant vNS2B-NS3-NS4A-NS4B-NS5 expressed the authentic NS3 and NS5 proteins, but the other recombinants produced uncleaved polyproteins. These findings indicate the NS2B is required for processing of the downstream nonstructural proteins, including the NS3/NS4A and NS4B/NS5 junctions, both of which contain a dibasic amino acid sequence preceding the cleavage site. The flavivirus NS4A/NS4B cleavage site follows a long hydrophobic sequence. The polyprotein NS4A-NS4B-NS5 was cleaved at the NS4A/NS4B junction in the absence of other dengue virus functions. One interpretation for this finding is that NS4A/NS4B cleavage is mediated by a host protease, presumably a signal peptidase. Although vNS3-NS4A-NS4B-NS5 expressed only the polyprotein, earlier results demonstrated that cleavage at the NS4A/NS4B junction occurred when an analogous recombinant, vNS3-NS4A-84%NS4B, was expressed. Thus, it appears that uncleaved NS3 plus NS5 inhibit NS4A/NS4B cleavage presumably because the putative signal sequence is not accessible for recognition by the responsible protease. Finally, recombinants that expressed an uncleaved NS4B-NS5 polyprotein, such as vNS4A-NS4B-NS5 or vNS4B-NS5, produced NS5 when complemented with vNS2B-30%NS3 or with vNS2B plus v30%NS3. These results indicate that cleavage at the NS4B/NS5 junction can be mediated by NS2B and NS3 in *trans*.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00597-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Construction and Characterization of Chimeric Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dengue virus, a flavivirus, causes human disease throughout subtropical and tropical regions of the world. There are four serotypes; infection with one produces durable immunity only to that type. Serotype is specified by the viral structural proteins. The dengue viral genome, an 11 kilobase strand of positive-sense RNA, contains of three structural protein genes followed by a series of 7 non-structural protein genes. Earlier, we constructed a full-length cDNA copy of the entire dengue 4 (D4) genome, RNA transcripts from which were infectious when transfected into mammalian cells. We subsequently replaced the structural protein genes of the full-length clone with the structural protein genes of D1 or D2 virus, and used these templates to create chimeric D1/4 and D2/4 viruses. The D4/D1 chimeric virus grows well in cells and makes the structural proteins of D1 virus. Two D4/D2 chimeric viruses have been made, one using a D2 virus isolated from a human patient, the other a mutant of that virus selected for mouse neurovirulence through serial mouse brain passage. The chimeric D4/neurovirulent D2 virus retained the neurovirulence property of its parent, but the level of neurovirulence was less than that of the parental type 2 virus. This indicated that at least some of the genetic loci responsible for neurovirulence are located in the structural protein genes. Sequencing of these genes in the neurovirulent D2 mutant and in its non-neurovirulent parent revealed 7 mutations resulting in amino acid changes. In order to determine which mutation(s) cause neurovirulence, I plan to make a series of chimeric viruses in which these mutations are isolated against a background of the prototype sequence. Elimination of the neurovirulence property may result in a chimeric D4/D2 virus safe for humans. Since the D4/D2 chimeric viruses appear to replicate more slowly than the parental viruses, they may also have properties of attenuation desirable in a vaccine. A set of suitably engineered chimeric viruses might serve together as a safe and effective tetravalent live dengue virus vaccine.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00598-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dengue Type 4 Virus Mutants Restricted in Polyprotein Processing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hiroshi Kawano, M.D.	Visiting Associate	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The dengue virus genome codes for a polyprotein in the order of NH_2-C-PreM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH that is proteolytically cleaved to generate the 10 individual proteins. Our previous studies indicated that cleavage between NS2B and NS3 is critical for the functional activity of the viral proteinase. Our goal is to elucidate the mechanism of polyprotein processing and apply the information to engineer stable dengue virus mutants that exhibit restriction of viral replication because a subset of such mutants should be less virulent than wild-type virus but still able to achieve a satisfactory level of immunogenicity during infection. This project was initiated to investigate the sequence requirements for proteolytic processing of dengue virus NS2B-NS3. Deletion analysis showed that two amino acids upstream of the NS2B-NS3 junction are sufficient for cleavage to take place. Subsequently, mutants of NS2B-NS3 containing single amino acid substitutions at the cleavage junction were constructed for analysis of their cleavage phenotype. Several substitutions resulted in an intermediate or a low level of cleavage. The finding that mutants of NS2B-NS3 containing substitutions at the cleavage junction exhibited a reduction of cleavage efficiency has provided a body of information for molecular analysis such as engineering of dengue virus mutants that are restricted in growth in a cell culture and perhaps in infected animals. Finally, full-length dengue type 4 cDNA constructs containing single amino acid substitutions at the NS2B-NS3 cleavage junction were prepared and used as templates for *in vitro* transcription. Recovered dengue virus mutants were verified for the presence of the mutant sequence in genomic RNA. Work is in progress to analyze the growth phenotype of these mutants. A series of deletion constructs of NS2A or NS2B that has been shown to affect poly protein processing will be employed to construct deletion mutants of dengue virus. Deletion mutants should be less subject to reversion of phenotype than amino acid substitution mutants. Future plans will include evaluation of virulence and immunogenicity of these mutants in infected animals.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00599-01 LID

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cleavage of the Dengue Virus Capsid Protein by the Viral Protease, NS3

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Others:	Barry N. Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

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  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED 1991-92

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-838

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00600-02 LID

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Engineering Viable Dengue Virus 3' Noncoding Region Deletion Mutants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ruhe Men, M.D.	Visiting Associate	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sequence analysis indicated that dengue type 4 viral genome contains 384 nucleotides in the 3' non-coding region (NCR). The last 82 nucleotides are thought to assume a secondary structure probably required for initiation of RNA replication. Preceding this structure are sets of conserved sequences, designated CS-1 and CS-2, that are also found in other mosquito-borne flaviviruses. We have engineered a series of cDNA constructs containing deletions ranging from 29 to 201 nucleotides in length in the 3' NCR. Mutant RNA transcripts made from these DNA constructs were tested for infectivity by transfecting permissive tissue culture cells. The last 113 nucleotides including the secondary structure at the 3' end and the preceding CS-1 sequence apparently are essential for infectivity of dengue virus. In contrast, viable dengue virus was recovered from mutant DNA constructs containing a deletion in one or the other of the two CS-2 sequences. Many viable deletion mutants constructed in this manner were stable and produced plaques of reduced size on infected C6/35 mosquito cells compared to wild type virus. This suggests that these mutants are restricted for their replication. These mutants are now being characterized further for their replicative capacity and other properties in cultured cells as well as in infected animals. Dengue virus mutants that show reduced virulence for animals will be evaluated subsequently in humans for evidence of attenuation and immunogenicity.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00637-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of New Approaches to Vaccines Against Tick-Borne Encephalitis Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alexander Pletnev, Ph.D., D.Sci.	Visiting Scientist	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Michael Bray, M.D.	Senior Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
Novosibirsk Institute of Bioorganic Chemistry; USAMRIID (Huggins)

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Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dengue type 4 virus (DEN4) cDNA was used as a vector to express genes of the distantly related tick-borne encephalitis virus (TBEV). Full length chimeric TBE/DEN4 cDNAs were constructed by substituting TBEV genes coding for proteins such as capsid (C), premembrane (M), envelope (E), or nonstructural protein NS1 for the corresponding sequences of DEN4. Full length RNA transcripts prepared from these cDNAs were used to transfect permissive simian cells. Two viable chimeric viruses that contained TBEV CME or ME genes were recovered. Compared to DEN4, chimeric TBE(ME)/DEN4 virus grew to higher titer and produced larger plaques in simian cells. In contrast, chimeric TBE(ME)/DEN4 virus produced smaller plaques on mosquito cells and grew to lower titer than DEN4. Analysis of viral RNA and proteins produced in TBE(ME)/DEN4- and DEN4-infected mosquito or simian cells revealed that the chimera was restricted in its ability to enter and replicate in mosquito cells. In contrast, TBE(ME)/DEN4 entered simian cells efficiently and its viral RNA was replicated more rapidly in these cells than was parental DEN4 viral RNA. Following intracerebral inoculation TBE(ME)/DEN4 virus caused fatal encephalitis in both suckling and adult mice, while mice inoculated by the same route with DEN4 did not develop disease. However, unlike wild-type TBEV, chimeric TBE(ME)/DEN4 did not cause encephalitis when adult mice were inoculated by a peripheral route. Adult mice previously inoculated with the chimera by a peripheral route were completely resistant to subsequent intraperitoneal challenge with 10^3 LD_{50} of TBEV, while mice previously inoculated with DEN4 were not protected. These findings indicate that: (i) the TBEV M and E genes of the chimeric virus are major protective antigens and induce resistance to lethal TBEV challenge, and (ii) other regions of the TBEV genome are essential for the ability of this virus to spread from a peripheral site to the brain. Success in constructing a TBE/DEN4 chimera that retains the protective antigens of TBEV but lacks its peripheral invasiveness provides a new strategy for the development of live attenuated TBEV vaccines.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00638-01 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Dengue and Other Flavivirus Proteins Using Baculovirus as a Vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chun-Fa Zhang, Ph.D.	Visiting Associate	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*
WRAIR, Washington, DC (Summers, Eckels, Dubois, Sumiyoshi)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The use of subunit proteins in dengue immunoprophylaxis offers a number of theoretical advantages, but this strategy has not been adequately explored. The baculovirus-insect cell system has been widely used to produce protein products of foreign genes, because: (1) a high level of expression of the foreign gene can be achieved, and (2) in a number of instances the insect cell system can process and modify expressed products to yield functionally and immunologically active proteins. Recent studies of vaccinia virus expressed dengue virus envelope protein (E) showed that a C-terminally truncated E, approximately 80% in length, is more immunogenic and protective than the full-length E in mice. The increase in immunogenicity and efficacy associated with the specific truncation of E has implications for the development of effective subunit vaccines for dengue as well as other flavivirus diseases. For these reasons, we constructed a total of 8 recombinant baculoviruses that expressed 80% E or full-length E of 3 different dengue virus serotypes (type 4, 2, and 3) and Japanese B encephalitis (JE) virus. The baculovirus-expressed 80% E proteins of dengue type 4, 2, 3 and JE viruses was secreted into the medium fluid of the infected cells and was stable. Most animals immunized with the intracellular form of 80% E or the control 93% E developed signs of encephalitis but none succumbed to the lethal challenge. On the other hand, none of the mice immunized with extracellular forms of 80% E showed encephalitic symptoms following dengue virus challenge suggesting that these animals developed a higher level of resistance. Analysis of sera from immunized mice confirmed that extracellular 80% E induced a 3- to 5-fold higher antibody response than that of intracellular 80% E of 93% E. Similar results were obtained in the case of dengue type 2 and 3. Immunization of rhesus monkeys with these E proteins expressed by recombinant baculoviruses is in progress.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00370-10 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Simian Immunodeficiency Virus (SIV) Infection of Macaques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Vanessa Hirsch, D.V.M., D.Sci.	Visiting Scientist	LID, NIAID
Others:	Simoy Goldstein, Ph.D.	Expert	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Tiffany Hamm	Biologist	LID, NIAID

COOPERATING UNITS *(if any)*
LIG, NIAID, NIH, Bethesda, MD (Fauci, Koenig); Georgetown University (Fomsgaard, Gerin, Johnson, London); Delta Primate Center (Murphy-Corb); Southwest Foundation (Allen)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Immunodeficiency Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	4.0	4.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Major emphasis was given to: (1) phylogenetic analysis of simian immunodeficiency virus (SIV) strains of African origin, (2) elucidation of viral determinants of SIV pathogenicity, and (3) evaluation of various SIV vaccine strategies.

An SIV isolate from a Sykes monkey was characterized and shown to be the prototype of a new, distinct primate lentivirus group.

Genetic determinants of the acutely lethal SIVsm/PBj mutant were identified using chimeric viruses produced by exchange of segments of this mutant and the minimally pathogenic SIVsm H4 clone. Virulence of SIVsm/PBj is complex and multigenic, involving regions of the envelope glycoprotein (*env*) as well as regions of *gag* and/or *pol*. One of five full-length molecular clones of SIVsm derived from an infected monkey that developed AIDS proved to be infectious. This clone will be studied in detail during the coming year. SIVsm clones with distinct cell tropism were used to produce infectious chimeras. Analysis of these chimeras indicated that macrophage tropism resides in the V3 loop region of *env*. Finally, an intensive analysis of virologic and immunologic events during the very earliest stage of SIV infection *in vivo* was initiated.

Our initial effort to develop an inactivated SIV vaccine was highly successful. Monkeys vaccinated with a psoralen-inactivated SIV suspension, derived from a proviral molecular clone, were completely resistant to challenge with homologous as well as heterologous (20% *env* sequence divergence) cell-free virus. IgG purified from the plasma of vaccinated monkeys protected naive monkeys from cell-free homologous SIV. However, the vaccine did not protect against challenge with cell-associated SIV but did appear to slow progression of disease.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00616-02 LID

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Feline Immunodeficiency Virus Infection as a Model for Human AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Raymond Langley, Ph.D.	Visiting Associate	LID, NIAID
Others:	Vanessa Hirsch, D.V.M., D.Sci.	Visiting Scientist	LID, NIAID
	Diane Adger-Johnson	Biologist	LID, NIAID
	Robert Goeken	Biologist	LID, NIAID

COOPERATING UNITS *(If any)*
Georgetown University (Johnson, Hirsch, Goff, Albert); NCI (O'Brien); Florida Fresh Water Fish and Game Commission (Roelke); Center for Retroviral Research, Ohio State University (Lafrado)

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Laboratory of Infectious Diseases

SECTION
Immunodeficiency Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Feline immunodeficiency virus (FIV) is a lentivirus that is associated with an immunodeficiency syndrome in naturally infected domestic cats. FIV was originally isolated from a feline leukemia virus (FeLV) negative cat at the University of California; this isolate designated FIV-Petaluma has become the prototype strain of FIV and was the original strain studied in this laboratory. A small animal model such as FIV infection of cats would be highly valuable as a model for the testing of antivirals and vaccine strategies. Therefore, our goals were to develop FIV infection of the cat as an experimental model for the study of lentiviral pathogenesis and AIDS. The specific objectives of this study were: (1) the molecular and biochemical characterization of FIV-Petaluma, (2) the infection of specific pathogen-free or domestic cats with FIV and a detailed analysis of the clinical response of infected cats, (3) an analysis of the genetic diversity of FIV isolates, (4) the development of reagents for vaccine trials, and (5) the search for FIV-related lentiviruses in wild cats. The major goals of this project in the past year have been the molecular characterization of a lentivirus isolated from Florida panthers, a wild cat that inhabits the Florida Everglades. The entire sequence of this panther lentivirus (PLV) was derived from PCR generated clones that span the genome. PLV is distantly related to FIV from domestic cats and shares a similar genome structure. The relationship between PLV and FIV is similar to that observed for different subgroups of the primate lentiviruses; the regulatory genes are poorly conserved, whereas the *gag* and *pol* proteins share 52 and 60% amino acid identity, respectively.

PHS 6040 (Rev. 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-938

LABORATORY OF IMMUNOGENETICS

ANNUAL REPORT

October 1, 1991 to September 30, 1992

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LABORATORY OF IMMUNOGENETICS

National Institute of Allergy and Infectious Diseases
October 1, 1991 to September 30, 1992

RESEARCH PROGRESS

Studies in the Laboratory of Immunogenetics continue to emphasize genes involved in immune function and molecular mechanisms involved in responses to immunization and infection. Novel findings have resulted from genetic mapping studies of the human TCR gene complex, and observations of genetically determined regions of insertion/deletion similar to those in the TCR complex have been observed in genetic maps of the rabbit MHC. Experiments involving infection of the rabbit with human retroviruses, HTLV-I and HIV-1, may give important clues to the mechanisms by which HTLV-I causes disease in certain individuals but not in others. Molecular dissection of antigen processing has shed new light on the early cellular events crucial to mounting an immune response. Studies on natural killer cells indicate that a novel cell surface molecule may be involved in directing their cytolytic activity. Studies continue on T-cells in the mouse that have suppressor or activator activity *in vivo.*

Studies of the Human T-cell Receptor Complex

Studies concerning the polymorphism of genes in the human TCR complex and the role of gene variation in normal immune responses and disease susceptibility have advanced in the past year. Progress toward a definitive map of the TCR genes was made showing that a previously described region of genetically determined insertion/deletion (IDRP) in the V gene complex contains TCR genes that may be replicates of genes that are encoded at other locations within the complex. One gene is a precise duplicate of a Vbeta13 gene that is also present in deleted TCRbeta haplotypes and the other is a previously uncharacterized member of the Vbeta7 family. Recent mapping data further indicate that certain V genes are not encoded within the TCR complex on chromosome 7. An additional cluster of Vbeta genes was localized to chromosome 9 by *in situ* hybridization and by use of a somatic cell hybrid panel. These findings that polymorphism includes presence or absence of certain TCR genes and that certain Vbeta genes are not within the complex have important implications to arguments about TCR gene expression in normal responses or in various autoimmune states. (**Robinson, Day, Zhao, Barron**).

Quantitative studies of TCR V gene expression have shown that TCR beta gene usage is not proportionate to the number of V genes present in the genome. The extent of the TCR repertoire was investigated by analysis of genomic and cDNA libraries prepared from lymphocytes of members of families well characterized for HLA. These studies revealed both extensions and limitations of TCR Valpha and Vbeta repertoires. The known repertoire has been extended by identification of new Valpha and Vbeta families, new members of defined families and by descriptions of allelic polymorphism in V gene segments. Limitations of TCR repertoires are imposed by the fact that certain V genes have been characterized as pseudogenes. The pseudogenes include an allelic variant of the Vbeta12.X gene that has a single nucleotide substitution resulting in a stop codon and thus is a non-functional allele as well as six Vbeta genes that are encoded outside of the gene complex and not observed in rearranged transcripts. Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states.

Studies of the Rabbit MHC

Molecular genetic analyses of the rabbit MHC (RLA) using pulsed field gel electrophoresis have shown that the relative order of class II genes (DP, DO, DQ, DR) is identical to that in humans and similar to that in mouse (**Chouchane**). However, a major difference from either HLA or H-2 was observed at the DR end of the RLA class II complex where class I genes are located in close proximity to DR with no interposed class III sequences. A fragment of 180 kb hybridized with the DR probe as well as with different class I probes including that for pR27, a class I gene with T-cell limited pattern of expression. Comparison of two different RLA haplotypes, A and B, indicated that the distance between the DQ and DR subregions differs by approximately 700 kb in the two haplotypes. Testing other unrelated rabbits suggested that this difference segregates within the rabbit population and presumably derives from an insertion/deletion event in different haplotypes. The region involved in the IDRP found for the RLA complex is analogous to that in which the genes for peptide transporters have been found in human. The probes for these genes are being prepared to explore the exciting possibility that the IDRP will involve insertion or deletion of genes with a vital immune function.

One gene identified in the rabbit MHC is the DOB gene which was first described for the HLA complex by Eric Long in this lab. *In vivo* expression patterns that can be easily determined in the rabbit may give some clue to the function of this unusual class II gene. As in human, the DOB gene in rabbit is highly conserved. The rabbit homologue of the human MHC-DOB gene was obtained from a rabbit spleen cDNA library using an HLA-DOB probe. The amino acid sequence deduced from the DNA sequence showed the typical structure of a class II beta chain and revealed that the rabbit DOB is closely related to HLA-DOB and to H-2 I-Abeta2, having 74% amino acid identity to HLA-DOB and 67% to the mouse gene product. Restriction fragment length polymorphism analysis of the rabbit DOB gene showed extensive polymorphism compared to that seen for HLA-DOB. However, sequence analyses of DOB from different rabbit MHC haplotypes indicated that exon 2 of the DOB gene, which encodes the beta1 domain, is highly conserved in all haplotypes tested. This finding, along with mapping studies which show a large region of insertion/deletion between DOB and DPA1 in different rabbit MHC haplotypes, suggest that RFLP patterns reflect structural differences in sequences flanking the DOB gene. Northern blot analysis of different rabbit organs and lymphoid cell populations indicated that the DOB gene has an expression pattern distinct from other class II genes and is expressed only in spleen, thymus, appendix and to a lesser extent in lymph nodes. The availability of this clone will allow preparation of antibodies to carry out a moreextensive study of the gene product.

HTLV-Infection in Rabbits Results in ATLL-like Disease

Human T cell lymphotropic virus-I (HTLV-I) is a retrovirus which is the etiologic agent of human adult T cell leukemia and the neurologic disorder termed Tsp/Ham. In addition, various other human disease conditions have been associated with HTLV-I infection. The majority of HTLV-I infected individuals, however, do not show disease signs. Although hypotheses concerning the progression of HTLV-I infection to disease have been advanced, no clear explanation for the myriad activities of this virus is available. Structural studies of HTLV-I reveal a highly conserved sequence for isolates taken from diverse populations and from individuals with different disease manifestations. As opposed to HIV and SIV sequences which vary enormously, HTLV-I sequences seldom diverge more than several percent. No infectious molecular clones of HTLV-I have been reported, therefore it is not yet possible to relate biologic activity to the observed structural variation. Although the rabbit can be readily infected with HTLV-I, the utility of the rabbit infection has been limited by the fact that, as in human infection, HTLV-I only causes disease in rare instances.

In our studies, a series of in vitro derived HTLV-I cell lines were used to carry out in vivo infection of NZW rabbits (**Sawasdikosol**). The majority of HTLV-I lines obtained, as well as the human or rabbit progenitors of these lines, mediated no disease in injected animals. Seroconversion and chronic cell infection were the only consequences even in rabbits injected with as many as 5 x 10^8 live HTLV-I infected cells. However, injection of one of the lines, RH/K34, caused rapid death when doses greater than 2 x 10^8 cells were given. Samples taken at necropsy showed evidence for leukemia-like infiltates of atypical cells in major organs (**Simpson**). This action of RH/K34 appeared to be independent of genetic relationships between the donor and recipient rabbits. Injection with irradiated RH/K34 cells also resulted in leukemia-like lesions similar to those caused by live cells, but did not cause the death of the injected rabbits.

The HTLV-I provirus was cloned from RH/K34 and compared to that from another cell line (RH/K30) obtained in a similar fashion but using the human cell line MT2 as source of virus (**Zhao, Robinson**). Both of the rabbit HTLV-I lines produce HTLV-I particles and are infectious for rabbit PBMC. The lines are morphologically distinct and show some variation in expression of cellular genes. However, unlike RH/K34 cells where injection into rabbits causes death with ATLL symptoms within several days, injection of RH/K30 cells in similar, or in even larger doses, has no overt effect. Structural comparison of virus cloned from the two lines was carried out in order to obtain information concerning differences which may elucidate their different properties and, by extrapolation, shed light on the varied actions of HTLV-1 in human infection. The viral sequences from these two lines were highly conserved, there were differences in both the 5' and 3' LTR and the *gag* gene of RH/K30 contained a 9 base insertion. In addition, both K34 and K30 viruses had numerous differences when compared to protypic sequences from HTLV-I infected human lines. Cloned virus was transfected into human and rabbit cell lines; rabbit cells transfected with K30 produced gag protein and some particles were seen by EM analysis. No transfections of human cell lines with K30, nor any transfection of rabbit or human cells with K34, yielded production of viral products. Continued studies of the clones and of the two cell lines may shed light on the differential ability of HTLV-I to cause human disease.

Infection of Rabbits with HIV-1

It was shown previously that laboratory rabbits may be infected with HIV-1 by administration of a single dose of infected human T cells. Recent studies of this potential animal model for AIDS have taken several new directions. In vivo studies have concentrated on the time course of infection and preliminary studies of drug and vaccine strategies to combat infection have begun. Cellular studies have targeted possible cell surface receptors for HIV-1 including the rabbit homolog of human CD4. Characterization of viral transcripts are underway in an effort to determine mechanisms for rabbit cell infection and to reveal means to enhance infectivity in these cells.

PCR analysis of samples from rabbits taken at necropsy showed that brain, spleen and other lymphoid tissue are heavily infected, whereas other tissues, such as liver, do not contain detectable levels of viral DNA or RNA. Peripheral blood lymphocytes from infected rabbits were tested for HIV-1 infection but virus was not detected until approximately 25 weeks post infection. Attempts to hasten this time are being made. An improved PCR reaction has increased sensitivity and a systemic study of the synergy of HTLV-I superinfection with HIV-1 infection has been carried out. Most promising have been experiments in which lymphoid tissues from rabbits infected for long periods of time were passaged to uninfected rabbits. The recipients showed the appearance of virus in peripheral cells as early as six weeks following administration of spleen cells from a rabbit that had been infected with HIV-1 two years previously.

Rabbit CD4 and HIV Infectivity *in vitro*

Susceptibility of rabbit cell lines to infection with HIV-1 raises questions concerning whether a CD4 homolog serves as HIV-1 receptor on rabbit cells. The rabbit CD4 homolog (RbCD4) was cloned from a rabbit thymus cDNA library; the deduced amino acid sequence was more similar to human and non human primate CD4 than to those of rodent and cat (**Hague**). However, six of the eighteen residues implicated in HIV-1 binding by CD4 differ between the human and rabbit proteins. RbCD4 expression was detected in thymus, lymph nodes and spleen by Northern blot analyses. No correlation between RbCD4 expression by rabbit cell lines and their ability to support HIV-1 infection was seen. Certain HTLV-I transformants showed high productivity of the viral gag protein, p24, upon HIV-1 infection even though the RbCD4 transcript levels were too low to detect by the PCR. Transfection of RbCD4 negative, HTLV-I transformed cell lines with HuCD4 significantly enhanced HIV-1 infectivity. This suggests that these lines are susceptible to infection, but lack a receptor present on other RbCD4 negative lines that produce high levels of p24 in their native state. HuCD4 transfection of a

RbCD4 positive line had little effect on HIV-1 infection suggesting that infection is not limited by viral attachment in this line. Significant inhibition of HIV-1 infection with soluble HuCD4 was demonstrated for all rabbit lines tested, but complete inhibition was obtained only with a rabbit T-cell line expressing RbCD4 and with HuCD4 transfectants. Virus titration on normal and transfected rabbit cells indicates that the relative affinity of receptors on rabbit cells is not equal to that of HuCD4. The results suggest that HIV-1 infection of the RbCD4 positive line proceeds through a receptor similar to HuCD4 but that an additional receptor or receptors may serve this purpose in RbCD4 negative lines.

In vitro studies of rabbit cell infection examined nef gene transcripts and protein produced in the rabbit cell lines compared to those produced in human cells infected with the same viral stocks (**Cho**). A study of the production of this regulatory protein indicated that about 15% of infected animals made antibody to it. It was shown that nef and other regulatory messages are present in low amounts in the rabbit cells relative to the human (**LeGuern**). Attempts will be made to carry out transfections with constructs making the regulatory protein in order to determine whether increased production of these may enhance the infection process in the rabbit. It is anticipated that *in vitro* results will have an impact upon future use of the rabbit infection model. For example, one may consider the use of a transgenic rabbits expressing human CD4 and/or HIV regulatory proteins in a cell specific manner.

Molecular Mechanisms of Antigen Presentation

These studies aim to define the function of human class II molecules in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens to CD4-positive T cells. Antigen processing for presentation by class II molecules generally involves endocytosis of antigen into an acidic compartment through which newly synthesized class II molecules travel on their way to the cell surface. Once at the cell surface, the foreign peptide/MHC class II complex interacts with the T cell receptor and the CD4 molecule expressed on the antigen-specific T cell. It was demonstrated here that several endogenous processing pathways lead to the presentation of cytosolic antigen to class II-restricted T cells. Most interestingly, one of the processing pathways was different from the one utilized for presentation by class I molecules. This unsuspected presentation of endogenous proteins by class II molecules has important implications for selection of the T cell repertoire, for T cell tolerance, and for autoimmunity. Several staphylococcal toxins bind to class II molecules and have a strong mitogenic effect on T cells by stimulating entire families of T cells with particular Vbeta chains in their TCR. These toxins are representative of the newly described superantigens that do not require processing for presentation. The invariant chain, bound to newly synthesized class II molecules, serves as a chaperone for the transport of class II molecules to an endocytic compartment where it dissociates from class II prior to the binding of immunogenic peptides and the cell surface appearance of peptide/class II complexes. It had been suggested that superantigens utilized the invariant chain binding site of class II molecules. However, our data have shown that the binding site of HLA-DR for the seven toxins tested was distinct from that for the HLA-DR-associated invariant chain. In addition, cell surface class II/invariant chain complexes were still able to stimulate T cells by superantigen. An important implication from this finding is that endogenous superantigens encoded by pathogens may bind to class II molecules intracellularly prior to the dissociation of the invariant chain (**Malnati, Roche, Pinet, Guéguen**).

NK Cell Specificity

Natural Killer (NK) cells are thought to play a role in the control of viral infection before the establishment of a specific cytolytic T cell response mediated by CD3-positive MHC-restricted T cells. Recently, specific recognition of alloantigens by NK cells was reported. Most of the cells displaying NK activity belong to the $CD3^-CD4^-CD8^-CD16^+CD56^+$ subset of peripheral blood lymphocytes. The aim of this study is to determine the degree of target cell specificity displayed by NK clones, and to eventually define the mechanism of target cell recognition by NK cells. As a first step, it was established that NK clones from a normal individual were able to specifically recognize and kill normal cells from the same individual that had been infected *in vitro* with Human Herpes Virus 6 (HHV6). However, only about half of the clones (58/118) were able to kill human Herpes virus 6 (HHV6)-infected autologous PHA-blasts, while all of them lysed the NK-sensitive cell line K562. A group of clones from two individuals were further characterized for their ability to recognize autologous and allogeneic infected cells. The results showed for the first time that specificity in target cell recognition by NK cells is controlled at several levels: first, at the level of the NK clone itself, and second, by genetically variable elements on the target cells. Because the cell surface level of class I MHC molecules was virtually unaffected by HHV6 infection, recognition by NK cells of these particular targets cannot be explained by the mere absence of class I molecules, as was proposed to explain the NK-mediated killing observed in other systems. Future studies are directed toward definition of the molecules involved in this critical first line of immune defense (**Malnati, Wagtmann**).

Cytokines Modulate the Antibody Response to SSS-III *in vivo*.

Previous studies demonstrated that suppressor T cells (**Ts**) can be influenced by cytokines. The present studies were designed to examine the effects of cytokines on amplifier T cell (**Ta**) activity by the administration of various cytokines at peak amplifier activity i.e. two days post-immunization with SSS-III. Treatment of mice with recombinant IL-6 or IL-5 increased the magnitude of the antibody response to SSS-III. Further, treatment with TGF b increased the secretion of IgA when this cytokine is given 2 days post-immunization. These findings suggest that the activity of both Ts and Ta cells is influenced by cytokines. (**Taylor, Baker**).

Modulation of Antibody Response to Pseudomonas LPS by IL-4.

Previous studies showed that the predominant IgG isotype produced in response to *Pseudomonas aeruginosa* LPS (PALPS) is IgG_3. We showed recently that when recombinant IL-4 is given all IgG isotypes (and IgM) are enhanced. Likewise, when anti-IL-4 antibody (11B11) is given the PALPS-specific antibody response was decreased for each isotype. However, treatment with 11B11 antibody increased total IgM synthesis, without affecting total IgG. While it is clear that

IL-4 can influence the antibody response to PALPS the underlying mechanisms remain to be resolved. (**Taylor, Baker**).

LABORATORY OF IMMUNOGENETICS
ANNUAL REPORT
October 1, 1991 to September 30, 1992

HONORS AND AWARDS

Dr. Thomas Kindt, Chief of the Laboratory of Immunogenetics, was an invited participant in a meeting on pathology and HIV infection in Hamburg, Germany. He was also invited to chair a workshop on animal models for AIDS at the International Congress of Immunology in Budapest, presented one of the keynote addresses at a meeting on biosafety and HIV/retrovirus research in Durham, North Carolina and presented laboratory data at the meeting of the Laboratory of Tumor Cell Biology in Washington, D. C. In addition, laboratory data were presented at the University of Connecticut, at the CDC in Atlanta, at Wright State University in Dayton, Ohio, at the meeting of the Experimental Immunology Branch of the NCI, at Hybridtech Incorporated and at the Bristol-Meyer Scribb Institute in Seattle. Members of Dr. Kindt's research section also presented data at the meeting of the FASEB in Anaheim in California. In July of 1992, Dr. Kindt finished a five year term as Deputy Editor of the *Journal of Immunology* and a 15 year term as Associate Editor of the *Journal of Experimental Medicine*. At the same time he was appointed Associate Editor of the *FASEB Journal* and remains North American regional editor of *Research in Immunology*. Dr. Kindt serves on the Board of Scientific Visitors for the Oklahoma Medical Research Foundation in Oklahoma City and serves in a similar capacity for the Research Program at the Virginia Mason Institute in Seattle, Washington. The Multiple Sclerosis Society awarded Dr. Kindt their Hope Award for completion of a six-year term as reviewer for research projects for the Multiple Sclerosis Society. Dr. Kindt continues to serve on the NIH Allergy and Immunology Study Section and serves on the scientific advisory boards of Oncor Inc., Gaithersburg MD, Innovir Laboratories Inc., New York, NY and served an ad hoc term on the SAB of TSI Inc, Worcester, MA.

Dr. Phillip Baker presented lectures and seminars at several universities and medical schools. He is on the Editorial Board of the ASM News, serves on the Public Relations Committee of the American Society for Microbiology (ASM) and also serves on the Publications Board of the International Endotoxin Society (IES). He was invited to give the Board of Education and Training Lecture, "Update '92 in Regulatory T Lymphocytes", at the annual meeting of the ASM in New Orleans, Louisiana.

Dr. Christopher Taylor was invited to convene a symposium on "Mechanisms of protective immunity: cytokines and isotype-specific antibody responses" at the annual meeting of the American Society for Microbiology, in New Orleans, LA. As chairman of the Immunology Division of the ASM, he organized a symposium on "Endotoxin effects on signal transduction" for the ASM meeting. Also, he represented NIAID at Dunbar High School, Washington, D. C., in April 1992 as part of the" NIAID's Adopt a School Program." He also participated in NIAID's "Introduction to Biomedical Research Program" in February, 1992.

Dr. Mary Ann Robinson presented an invited lecture at Georgetown University, Department of Pathology in February. She served on the Abstract Selection and Awards Committees for the American Society of Histocompatibility and Immunogenetics and chaired a session on T cell Receptors and Immunomodulation at the 1991 annual meeting in Washington, D. C. Dr. Robinson also served on the selection committee for NIAID's Introduction to Biomedical Research and presented at the round table discussion. Dr. Robinson was invited to join the Editorial Board of the journal *Human Immunology*.

Dr. Eric Long presented seminars at Emory University in Atlanta, at the Laboratory of Biochemistry at NCI, NIH, at the University of Geneva and the University of Lausanne, Switzerland, and at the University of Wisconsin, Madison. Dr. Long also received the NIH Director's Award in June 1992.

LABORATORY OF IMMUNOGENETICS

ANNUAL REPORT

October 1, 1991 to September 30, 1992

ADMINISTRATIVE REPORT

The Laboratory of Immunogenetics has spent its first full year at the NIAID Twinbrook II facility in Rockville, Maryland. Laboratories and animal spaces in the building are fully operational and the laboratory continues to maintain a contract at Spring Valley Laboratories for animals infected with human retroviruses. Dr. Kindt has been active in operations of the Twinbrook II facility including recruitment for the NIAID Structural Biology Laboratory and in design of new offices and laboratory space. The Immunogenetics Research Section of the laboratory was joined in the spring of this year by Dr. Karyl Barron who jointly holds a position in the Department of Medicine at Children's Hospital in Washington, D. C. Dr. Barron will work with Dr. Mary Ann Robinson. In addition, Dr. Awatef Ismail, from Tunisia, will join the laboratory in September as will Dr. Michel Leno from the Institut Pasteur in Paris. In the past year, Dr. Annie LeGuern, a Visiting Associate, left the laboratory to assume a position as a Research Scientist in Biotransplant Incorporated in Charlestown, Massachusetts. Dr. Mark Gordon left the laboratory position of Staff Fellow to assume a position with a new biotech company that will be located in Woodbine, Maryland. The Molecular Immunology Section of the laboratory was joined by Dr. Maryse Gueguen from the University of Paris, who will work with Dr. Eric Long.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00143-23

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Control of the Antibody response to Microbial Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P.J. Baker, Ph.D., Head, Microbiology & Immunology Section, LIG, NIAID
Other: C.E. Taylor, Ph.D., Senior Staff Fellow, LIG, NIAID
T. Hraba, Ph.D., Visiting Scientist, LIG, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE DURING YEAR.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00144-28

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P.J. Baker,Ph.D Head, Microbiology & Immunology, LIG, NIAID
Other: C.E. Taylor, Ph.D., Senior Staff Fellow, LIG, NIAID
T. Hraba, Ph.D., Visiting Fellow, LIG, NIAID
K.R. Haslov*, P. Ibsen*
A. Fomsgaard**, J.S. Fomsgaard**
K. Takayama***

COOPERATING UNITS *(if any)*

*Vaccine Dept., Statens Seruminstitut, Copenhagen, Denmark; **Dept of Clinical Microbiology, Rigshopitalet, Copenhagen, Denmark; ***Mycobacteriology Laboratory, William S. Middleton Memorial VA Hospital, Madison WI

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunomodulatory properties of several lipopolysaccharides (LPS) derived from clinical isolates of Pseudomonas aeruginosa, Branhamella catarrhalis, and Bordetella pertussis were evaluated for their capacity to influence the magnitude of the antibody response to type III pneumococcal polysaccharide (SSS-III), which is known to be regulated in a negative and positive manner by suppressor and amplifier T cells (Ts and Ta, respectively). The administration of LPS, two days after immunization result in a significant increase in the antibody response. Such enhancement appears to be due to the ability of the lipid A moiety of LPS to abolish the negative effects of activated Ts, thereby enabling Ta function to be more fully expressed. By contrast, treatment with LPS at the time of immunization with SSS-III-specific i.e., prior to the activation of Ts induces significant suppression of the SSS-III-specific antibody response, such suppression is independent of the capacity of LPS to activate B cells polyclonally, an activity generally attributed to the lipid A fraction of LPS. Studies conducted with LPS of P. aeruginosa indicated that the suppression induced is T-cell dependent and mediated by the polysaccharide (PS) fraction of LPS: it appears to be due - at least in part - to the capacity of PS to expand or increase the size of the precursor pool of Ts, activated in response to SSS-III. These findings suggest that the capacity of the PS fraction of LPS to augment Ts function may play an important role in the pathogenesis of certain gram-negative infections.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00145-24

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.J. Baker,	Head, Microbiology and Immunology Section, LIG, NIAID
Other:	C.E. Taylor,	Senior Staff Fellow, LIG, NIAID
	T. Hraba,	Visiting Scientist

COOPERATING UNITS *(if any)*

*K. Myers, Ribi ImmunoChem Res.,MT;**K. Takayama, N. Qureshi, Mycobacteriology Lab.,WI; ***P. Stuetz, Sandoz Forschungsinstitut,Austria; ****S. Kusumoto, A. Hasegawa, Osaka University, Osaka, Jpn; *****Gifu University, Gifu, Jpn.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lipid A preparations derived from the lipopolysaccharides of several gram-negative bacteria, as well as chemically defined synthetic lipid A's and their analogs (both glucosamine mono- and disaccharides), were used to establish the chemical structures required for (a) abolishing the expression of suppressor T cell (Ts) function, and (b) the ability to induce polyclonal activation of B cells. Salmonella minnesota R595 lipid A (diphosphoryl lipid A) possesses both of these activities. Decreasing the number of phosphate groups in lipid A from two to one (monophosphorly lipid A) as well as decreasing the fatty acyl content primarily by removing the residue at the 3-position resulted in a progressive reduction in toxicity; however, these structural modifications did not influence its ability to abolish the expression of Ts function. Reducing the fatty acyl content from five to four (lipid A, precursor IVA or Ia) eliminated its capacity to influence Ts function, but not to induce polyclonal activation of B cells. None of the monosaccharide analogs of lipid A examined influenced the expression of Ts activity, although some were able to activate B cells polyclonally. Thus, in order to be able to abolish the expression of Ts function, lipid A: (a) must be a glucosamine disaccharide; (b) may have either one or two phosphate groups; and (c) must have at least five fatty acyl groups. Also the chain length of the nonhydroxylated fatty acid, as well as the location of acyloxyacyl groups (2'- vs 3'-position) may play an important role. These findings indicate that the chemical structures responsible for the toxicity of lipid A differ from those that influence its capacity to abolish the expression of Ts function and to induce polyclonal activation of B cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00166-15

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T.J. Kindt, Chief, LIG, NIAID
Other: L. Chouchane, Visiting Fellow, LIG, NIAID

COOPERATING UNITS *(if any)*

P. Marche, Pasteur Institut, Paris, France and C. LeGuern, Trans Biology Unit, MA General Hospital, MA

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.25	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the genes of the major histocompatibility locus (RLA) of the rabbit have revealed major differences in the organization of these genes compared to other species. Molecular analyses of genes in the rabbit MHC (RLA) by pulsed field gel electrophoresis have shown that the relative order of class II genes (DP, DO, DQ, DR) is identical to that in humans and similar to that in mouse. However, a major difference from either HLA or H-2 was observed at the DR end of the RLA class II complex: class I genes are located in close proximity to DR with no interposed class III sequences. A further unique feature was found by comparison of two different RLA haplotypes, A and B. Mapping data indicated that the distance between the DQ and DR subregions differs by approximately 700 kb in the two haplotypes and this difference segregates within rabbit population. The large genetically determined difference presumably derives from an insertion/deletion even in different haplotypes. The RLA complex includes a class II beta chain similar to human MHC-DOB which has a conserved structure and expression pattern different from other class II genes. The rabbit DOB gene was obtained from a rabbit spleen cDNA library and the deduced amino acid sequence showed the typical structure of a class II beta chain; comparisons revealed that the rabbit DOB is closely related to HLA-DOB and to H-2 I-Abeta2, having 74% amino acid identity to HLA-DOB and 67% to the mouse gene product. Restriction fragment length polymorphism analysis of the rabbit DOB gene showed extensive polymorphism compared to that seen for the human HLA-DOB. However, sequence analyses of DOB from different rabbit MHC haplotypes indicated that exon 2 of the DOB gene, which encodes the beta1 domain, is highly conserved in all haplotypes tested. This finding, along with that of the insertion/deletion between DOB and DPA1 in different rabbit MHC haplotypes, suggest that RFLP patterns reflect structural differences in sequences flanking the DOB gene. A search for the functional role for DOB is underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00168-15

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. J. Kindt, Ph.D., Chief, LIG, NIAID
OTHER: Mark Gordon, Ph.D, Staff Fellow, LIG, NIAID
Bishop Hague, Ph.D., Staff Fellow, LIG, NIAID
Sansana Sawadikosol, Biologist, LIG, NIAID

COOPERATING UNITS *(if any)*

J.M. Wilkinson, Royal College of Surgeons, London

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.25	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiments are underway to characterize a number of markers for rabbit lymphoid cells. The major approach used is to clone the genes encoding cell surface molecules known to be involved in identification of functionally distinct cell subsets. These will be valuable in identifying distinct cell populations and in evaluating the expression of the genes in lymphoid tissues. These genes will be expressed for the production of monoclonal antibodies to the cell surface markers or as soluble forms of the cell bound products that may be used for inhibition studies. The gene for rabbit CD4 has been cloned and sequenced. It is being expressed as a membrane form on the cell surface as well as soluble form. The product of both expression systems can be used to determine the efficiency of HIV-1 binding to cells. The gene for rabbit CD8 is being cloned from a cDNA library. CD4 and CD8 identify important subpopulations of T cells. The genes for IL2, IL2R alpha and those for the gamma and delta chains of the T cell receptor are also under investigation. The molecular probes and other reagents derived from the genes can be used for monitoring these markers in the human retrovirus disease models being developed in the rabbit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00170-15-LIG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eric O. Long, Ph.D.	Visiting Scientist, LIG, NIAID
OTHER:	P.A. Roche, Ph.D	Staff Fellow, LIG, NIAID
	M. Malnati, M.D.	Visiting Fellow, LIG, NIAID
	V. Pinet, Ph.D.	Visiting Fellow, LIG, NIAID
	M. Gueguen, Ph.D.	Visiting Fellow, LIG, NIAID

COOPERATING UNITS *(if any)*

W. Biddison, Neuroimmunology Branch, NINDS, NIH; R. DeMars, University of Wisconsin, Madison, Wisconsin

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.7	4.3	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded by the major histocompatibility complex (MHC). These self molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules. Antigen processing for presentation by class II molecules generally involves endocytosis of antigen into an acidic compartment through which newly synthesized class II molecules travel on their way to the cell surface. Once at the cell surface the foreign peptide/MHC class II complex interacts with the T cell receptor and the CD4 molecule expressed on the antigen-specific T cell. The aim of this project is to define the function of human class II molecules in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens to CD4-positive T cells. It was demonstrated that several endogenous processing pathways lead to the presentation of cytosolic antigen to class II-restricted T cells. Most interestingly, one of the processing pathways was different from the one utilized for presentation by class I molecules. This unsuspected presentation of endogenous proteins by class II molecules has important implications on the T cell repertoire selection, on T cell tolerance, and on autoimmunity. Several staphylococcal toxins bind to class II molecules and have a strong mitogenic effect on T cells by stimulating entire families of T cells with particular Vbeta chains in their TCR. These toxins are representative of the newly described superantigens that do not require processing for presentation. The invariant (Ii) chain, bound to newly synthesized class II molecules, serves as a chaperon for the transport of class II molecules to an endocytic compartment where it dissociates from class II prior to the binding of immunogenic peptides and the cell surface appearance of peptide/class II complexes. It had been suggested that superantigens utilized the Ii chain binding site of class II molecules. However, it was shown that the binding site of HLA-DR for the 7 toxins tested was distinct from that for the HLA-DR-associated Ii chain. In addition, cell surface class II/Ii chain complexes were still able to stimulate T cells by superantigen. An important implication from this finding is that endogenous superantigens encoded by pathogens may bind to class II molecules intracellularly prior to the dissociation of the Ii chain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00171-15

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infection of Rabbits with Human Immunodeficiency Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.J. Kindt	Chief	LIG, NIAID
Other:	A. LeGuern	Visiting Associate	LIG, NIAID
	S. Cho	Visiting Fellow	LIG, NIAID
	R.M. Simpson	Expert	LIG, NIAID

COOPERATING UNITS *(if any)*

Mark Girard, Institut Pasteur, Paris

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.1	2.2	2.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Laboratory rabbits may be HIV infected by administration of a single dose of infected human T cells. Recent studies of this potential animal model for AIDS have taken several new direction. PCR analysis of samples from rabbits taken at necropsy showed that brain, spleen and other lymphoid tissues are heavily infected, whereas other tissues, such as liver, do not contain detectable levels of viral DNA or RNA. Peripheral blood lymphocytes from infected rabbits were tested for HIV-1 infection, but virus was not detected until approximately 25 weeks post-infection. Attempts to hasten this time are being made. An improved PCR reaction has increased sensitivity and a systematic study of the synergy of HTLV-I superinfection with HIV-1 infection has been carried out. Most promising have been experiments in which lymphoid tissues from rabbits infected for long periods of time were passaged to uninfected rabbits. The recipients showed the appearance of virus in peripheral cells as early as six weeks following administration of spleen cells from a rabbit that had been infected with HIV-1 for two years previously. A study of the production of the regulatory protein, nef, indicated that about 15% of infected animals made antibody to it. In vitro studies of infection of rabbit cell lines have focused on the nef gene transcripts and protein produced in the rabbit cell lines, and compared to those produced in human cells infected with the same viral stocks. It was shown that nef and other regulatory messages are present in very low amounts in the rabbit cells relative to the human. Attempts will be made to carry out transfections with constructs making the regulatory proteins in order to determine whether increased production of these may enhance the infection process in the rabbit. It is anticipated that in vitro results will have an impact upon future use of the rabbit infection model. For example, one may consider the use of a transgenic rabbit expressing HIV regulatory proteins in a tissue specific manner.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00180-14

PERIOD COVERED

October 1. 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.J. Kindt	Chief	LIG, NIAID
Other:	S. Sawasdikosol	Biologist	LIG, NIAID
	R.M. Simpson	Expert	LIG, NIAID
	B. Hague	Staff Fellow	LIG, NIAID
	T. Zhao	Staff Fellow	LIG, NIAID
	M.A. Robinson	Senior Investigator	LIG, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.3	2.9	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A series of transformed rabbit cells were developed in this laboratory by co-culture of human T-cell lymphotrophic virus type I (HTLV-I)-infected rabbit or human leukemia-causing cells with rabbit peripheral blood mononuclear cells. These cell lines are being used to study viral gene expression and regulation of cellular processes as well as to study mechanisms of disease caused by HTLV-I, the human leukemia virus. The series of cell lines produced include cells with diverse in vitro and in vivo phenotypic characteristics, even though similar methods and virus were used to create them. All cell lines had HTLV-I provirus that was integrated at various sites in the genome. All cell lines produced p19 viral core protein and many exhibited productive-appearing virions in cytoplasmic vesicles or in extracellular spaces, when examined by transmission electron microscopy. All cell lines were capable of infecting and transforming cells when co-cultured with fresh rabbit peripheral blood mononuclear cells (PBMC). All rabbit cell lines lacked the CD4 receptor (CD4-), had T-cell antigen receptor (TCR) gene rearrangements and lacked immunoglobulin (Ig) lambda light chain gene rearrangement. Some cell lines utilize alpha/beta TCR while others use delta/gamma chains. Despite all these classical T-cell characteristics, cells exhibited a spectrum of phenotypic characteristics (within and) among cell lines, including different morphology, lysosomal contents, phagocytic capability and characteristics of in vitro growth. Some of the cell lines were lethal when inoculated into rabbits while others were not. A CD4- cell line expressing beta/delta TCR transcripts caused leukemia and lymphoma that resulted in the death of rabbits in a dose dependent manner. This disease is a model for the acutely lethal stage of adult T-cell leukemia/lymphoma (ATLL) occurring in some humans infected with HTLV-I. Characterization of this disease model is underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00389-09-LIG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genotype Analyses of HLA and TCR Genes in Human Families

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Robinson	Senior Investigator	LIG, NIAID
Other:	C. Day	Senior Staff Fellow	LIG, NIAID
	T. Zhao	Staff Fellow	LIG, NIAID
	K. Barron	IPA	LIG, NIAID

COOPERATING UNITS *(if any)*

D. Bernard Amos, Duke University, N.C.; P. Concannon, Virginia Mason Institute, Seattle, WA; P. Lusso, NCI

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.1	3	2.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inheritance patterns of HLA and T cell receptor (TCR alpha and TCR beta) genes which are known to play important roles in a variety of immune processes have been analyzed in human families. The extent of the TCR repertoire was investigated by analysis of cDNA and genomic libraries prepared from DNA samples dreived from the members of families well characterized for HLA. The present studies have revealed both extensions and limitations of TCR V alpha and V beta repertoires. The known repertoire has been extended by identification of new V alpha and V beta families, new members of defined families and allelic polymorphism in V gene segments. On the other hand, certain V genes have been characterized as pseudogenes which limits TCR repertoires. Six V beta genes were found to be encoded on chromosome 9 outside of the TCR beta gene complex which is encoded on chromosome 7. These genes, designated orphons, have sequences 93-97% identical to V beta genes in the TCR beta gene complex, show no obvious defects that would preclude expression, but are not detected as rearranged transcripts with C beta. Two frequently occurring insertion/deletion related polymorphisms (IDRP) were found in the TCR beta gene complex; one involves a stretch of ~30kb in the V region and another spans ~20kb near the C region. TCR V beta genes have been localized to the V region IDRP; one gene is previously uncharacterized member of a V beta family and another is a precise duplication of a V beta gene also present in deleted TCR beta haplotypes. Utilization of TCR gene segment by peripheral T cells was found to be disproportional to gene number. Statistically significant skewing in the frequencies of V beta, J beta, and C beta gene segments present in TCR beta transcripts was observed. TCR V alpha genes were also found to be used in a non-random manner. Family studies have revealed that utilization of certain TCR V beta genes maps to the TCR beta complex, however, neither HLA nor TCR genes can explain skewed utilization of all TCR V gene segments. Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00525-05-LIG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Human Killer T cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. O. Long, Ph.D.	Visiting Scientist, LIG, NIAID
Other:	M. Malnati, M.D.	Visiting Associate, LIG, NIAID
	M. Wagtmann, Ph.D.	Visiting Fellow, LIG, NIAID

COOPERATING UNITS *(if any)*

P. Lusso, Lab. Tumor Cell Biology, NCI, NIH; A. and L. Moretta, National Institute for Cancer Research Genova, Italy

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.9	1.7	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Natural Killer (NK) cells are thought to play a role in the control of viral infection prior to the establishment of an MHC restricted CTL response. Recently, specific recognition of alloantigens by NK cells was reported. Most of the cells displaying NK activity belong to the CD3-CD4-CD8-CD16+CD56+ subset of peripheral blood lymphocytes. The aim of this study is to determine the degree of target cell specificity displayed by NK clones and to eventually define the mechanism of target cell recognition by NK cells. As a first step, it was established that NK clones from a normal individual were able to specifically recognize and kill normal cells from the same individual that had been infected in vitro with Human Herpes Virus 6 (HHV6). However, only about half of the clones (58/118) were able to kill human Herpes virus 6 (HHV6)-infected autologous PHA-blasts, while all of them lysed the NK-sensitive cell line K562. A group of clones from two individuals were further characterized for their ability to recognize autologous and allogeneic infected cells. The results showed for the first time that specificity in target cell recognition by NK cells is controlled at several levels: first, at the level of the NK clone itself, and second, by genetically variable elements on the target cells. Because the cell surface level of class I MHC molecules was virtually unaffected by HHV6 infection, recognition by NK cells of these particular targets cannot be explained by the mere absence of class I molecules, as was proposed to explain the NK-mediated killing observed in other systems.

LABORATORY OF IMMUNOPATHOLOGY

1992 Annual Report
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Summary Statement
LABORATORY OF IMMUNOPATHOLOGY
National Institute of Allergy and Infectious Diseases
October 1, 1991 to September 30, 1992

The primary programs in the Laboratory of Immunopathology are centered on host/virus interactions in relation to induction of neoplasia and/or immunodeficiency. Most studies have taken advantage of rodent model systems to study these effects with murine retroviruses and papovarviruses being the agents of greatest interest. Continuing emphasis is placed on studies of normal hematopoietic differentiation and the effects of mutated genes, particularly oncogenes, as they affect these processes. The major accomplishments of the Laboratory in pursuing these studies are as follows:

Retrovirus-induced immunodeficiency. A mouse disease, termed MAIDS, with many similarities to HIV-induced disease in man has been developed in LIP as a model for AIDS. This system has been used extensively to evaluate the nature of the murine leukemia viruses (MuLV) responsible for disease, the means by which the viruses affect the immune system including the nature of the immunodeficiency and to explore therapeutic modalities.

The etiologic virus in MAIDS was previously shown on biologic and molecular grounds to be a replication defective virus that expresses only a *gag* protein. The cloned genome of the defective virus (BM5def) has been engineered to express a bacterial neomycin resistance gene (BM5def/neo) to facilitate studies of the role of different helper viruses in disease induction and the cells targeted for infection and expression of the defective virus (Chattopadhyay, Morse, Hartley). These studies have shown BM5def/neo rescued by nonpathogenic ecotropic and amphotropic helpers to be biologically active and to induce disease indistinguishable from that induced by the uncloned virus mixture (Hartley, Chattopadhyay).

The *gag* gene of BM5def was also used to generate a transgene construct driven by immunoglobulin heavy chain enhancers (Chattopadhyay, Morse, Flavell). Mice bearing the transgene have been generated and are being tested for germline transmission and evidence of disease. These animals should prove to be extremely valuable for assessing the effects of uniform expression of the virus gene in cells of the B lymphocyte lineage and introducing the gene into different genetic backgrounds.

Previous studies suggested that *gag* protein of BM5def had features of a superantigen. This suggestion was strengthened by studies showing the involvement of particular T cell receptor Vb families in the response to the *gag* expresssed on a B cell tumor (Kanagawa, Morse) and the finding that T cells from mice with MAIDS are profoundly anergic (Hugin, Giese, Morse). Efforts to develop a new *in vitro* system for studying this superantigen include the generation of transfected B cell lines expresssing the *gag* protein (Kim, Chattopadhyay, Morse).

Enhanced susceptibility to exogenous infections and lymphomas was earlier shown to be a prominent feature of MAIDS. Only recently, however, has opportunistic infection resulting in death been observed, the agent being *Citrobacter freundii* with infection causing a severe ulcerative colitis and septicemia (Fredrickson, Morse, Hartley). Additional studies of the interactions between MAIDS and infectious agents showed that mice developed inclusion body disease induced by murine cytomegalovirus of greater severity and duration when infected with retrovirus for 6 weeks or more (Hartley, Morse, Fredrickson).

More detailed analyses of lymphomas occurring in MAIDS were also performed using adoptive transfer of cells to scid mice. Strikingly, infected mice were found to develop CD4$^+$ as well as B cell lineage lymphomas, all of which contained BM5def, with some tumors appearing as early as 9 weeks post infection. Also unexpected was the observation that spleens of single mice often contained two distinct tumors of T and B cell origin (Tang, Chattopadhyay, Morse, Fredrickson, Hartley). The lymphomas appearing in scid mice were clonal, as they were in donor mice, but in the recipients they were considerably more aggressive and invasive of nonlymphoid tissue. Unlike AIDS lymphomas, the _myc_ oncogene was not frequently altered in structure (Tang et al).

Finally, the MAIDS system was examined for the effects of combining anti-viral drugs with agents that inhibit the proliferative aspect of the disorder. These studies showed that AZT and cyclosporine produced an additive effect in slowing the progress of MAIDS with treatment initiated after infection (Cerny, Izui, Morse).

DNA tumor viruses. The Viral Pathogenesis Section has continued long term analyses of the mechanisms responsible for tumor induction by cells transformed by papovaviruses with emphasis on adenoviruses. These studies examine the ability of cells transformed _in vitro_ by papovaviruses to grow in hosts with varying levels of immune function, the spectrum including T cell deficient nude mice, newborn mice and adult immunocompetent animals. The assays are performed as cell titrations to permit determination of the threshold dose for tumor formation, the time required for tumors to develop and the incidence of tumors at each cell dose. The data from these tests have been subjected to a novel form of biometric analysis designed to permit the identification of distinct tumor cellular phenotypes controlled by different viral oncogenes. These analyses suggest that 4 independent viral gene functions contribute to the ability of cells transformed _in vitro_ to produce tumors (Lewis, Cook Alling).

To determine if these characterizations of cells transformed _in vitro_ relate to features of tumors induced _in vivo_, mice of various strains were infected with adenovirus strain 12 and observed for tumor development. Some strains of mice but not others developed tumors even though cells from all strains could be transformed _in vitro_. All the _in vitro_ transformed cells express low levels of class I MHC protein (Eyler, Lewis).

Oncogenes in B cell neoplasia. The CBL oncogene has been the major focus of study in this line of investigation. This gene, captured in a truncated form by an acutely transforming retrovirus, induces B cell lineage tumors and some myeloid leukemias. Efforts to establish the genomic structure of the gene have revealed that it is composed of multiple small exons spanning more 20 kb (Shapiro).

Analyses of CBL expression demonstrated that in spite of some structural similarities to immediate early genes, it is not expressed soon after FGF$^+$

serum stimulation of serum-starved fibroblasts or after mitogenic stimulation of T cells or B cells. However, expression was found to be reduced on induction of differentiation of F9 cells (Morse, Mushinski). Studies of CBL transcripts have revealed two or more alternative forms, one containing an aminoterminal leucine zipper and the second with no zipper (Shapiro, Morse). The significance of these differences isnot know, but efforts are being made to define proteins that may interactd with the zippered form of the protein (Shapiro).

Efforts to more clearly define the transforming potential of CBL have included the generation of mice bearing CBL in two different forms under the control of immunoglobulin heavy chain enhancers. One strain of mice bears a complete CBL cDNA and while containing multiple copies of the gene, there is no increase in CBL protein expression suggesting the genes are not transcribed or alternatively not translated (Campbell). The second set of transgenics bears CBL in a form resembling the viral truncation. These mice are under study for expression of the gene (Campbell, Jhappan). In addition, a series of viral constructs containing serveral forms of CBL, including varying deletions, are being tested for their ability to transform fibroblasts in vitro (Campbell, Langdon).

Hematopoietic differentiation. The major emphasis in this program has been to define the charateristics of B cell differentiation and activation using monoclonal antibodies (mAb). Analyses of the antigen recognized by the LIP-6 antibody indicate that it is involved in B cell activation as the mAb stimulates T cell independent proliferation of B cells (Holmes, Morse). Efforts to molecularly clone the gene have been initiated.

Another mAb developed in the laboratory, BRB-44, is highly polymorphic in its expression on cells of inbred strains and appears to be a marker, in low expressing strains, for activated T cells and B cells (Holmes). Expression is markedly increased on both cell types in mice with MAIDS (Holmes, Morse).

Finally, studies of the Ly-26 antigen suggest that it may be part of a multimeric complex on the cell surface that includes CD11b (Mac-1) (Holmes).

Administrative changes. Dr. Eggerton Campbell will be leaving the Laboratory to enter administration and Dr. Ambros Hügin will return to Switzerland to work in the Department of Medicine at the University of Geneva. Dr. Wan Kee Kim joined the Laboratory as a Visiting Fellow.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00013-29 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Andrew M. Lewis, Jr., Head, Viral Pathogenesis Section, LIP, NIAID
Others: Silvia Soddu, Visiting Fellow, LIP, NIAID
Yvonne Eyler, IRTA Fellow, LIP, NIAID
David Alling, Collaborator, ODIR
Steve Banks, Collaborator, ODIR

COOPERATING UNITS *(if any)*

National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado (J.L. Cook)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the past 4 years, we have developed a new method of studying the role of viral gene functions in the tumorigenicity of virus transformed cells. This method is based on quantitative analysis of the tumor inducing capacity of DNA virus transformed cells in animals that possess different capacities to mount cellular immune, antitumor defenses. For this method, the relationship between transformed cell dose, tumor incidence and time of tumor appearance (tumor latency) is determined repetitively for clones of rodent embryo cells transformed by different viruses. By expressing the transformed cell dose - tumor incidence data as the mean of the tumor producing dose 50% end point (TPD50) and the transformed cell dose - tumor latency data as the average tumor latency, an objective characterization of the tumorigenicity (i.e. tumor inducing capacity or tumorigenic phenotype) of any particular transformed cell clone can be obtained. Significant differences in mean TPD50 values or average tumor latencies of the same cells in different types of tumor challenged hosts identifies the specific traits of tumor cells that may be induced by viral gene functions. Differences in these data among cell lines transformed by different viruses in different types of hosts permits identification and classification of the different viral gene functions that are putatively involved in transformed cell tumorigenicity. Standard statistical analyses of such data have proven to be inadequate. Attempts are underway to develop alternative statistical approaches.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00135-16 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Properties of Immunoglobulin Secreting Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, Chief, LIP, NIAID
Others: U.R. Rapp, Senior Investigator, LVC, NCI
J.H. Pierce, Senior Investigator, LCMB, NCI
K.L. Holmes, Senior Investigator, BRB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE THIS YEAR 1991-92

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00138-15 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Viruses and Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, Chief, LIP, NIAID
Others: J.W. Hartley, Head, Viral Oncology Section, LIP, NIAID
K.L. Holmes, Senior Investigator, BRB, NIAID
T.N. Fredrickson, Expert, NCI
R.M.L. Buller, Senior Staff Fellow, LVD, NIAID
A. Singer, Chief, LEI, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE THIS YEAR 1991-92

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00205-09 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, Chief, LIP, NIAID

COOPERATING UNITS *(if any)*

Juntendo University, Tokyo, Japan (T. Shirai, K. Okumura)

LAB/BRANCH

Labroatory of Immunopathology

SECTION

Virology and Cellular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE THIS YEAR 1991-92

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00284-11 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Pathogenic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Janet W. Hartley, Head, Viral Oncology Section, LIP, NIAID
Others: H.C. Morse III Chief, LIP, NIAID
T.N. Fredrickson, Expert, NCI
S.K. Chattopadhyay, Expert, LIP, NIAID
Y. Tang, Visiting Associate, LIP, NIAID
T. Torrey, Senior Staff Fellow, LIP, NIAID

COOPERATING UNITS *(if any)*

National Institute of Health, Japan (M. Makino)
FDA (A. Rosenberg, A. Khan)
Laboratory of Neuroscience, NIDDK (Y. Sei)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	1.7	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project deals with the biological and molecular characterization of murine leukemia viruses (MuLV) with the goal of understanding host and viral factors involved in the pathogenesis of leukemias, lymphomas and a variety of non-neoplastic sequelae of infection with certain of these viruses. . The primary subject of current study is the complex of viruses (LP-BM5 MuLV) responsible for induction of murine AIDS (MAIDS). Infection of sensitive strains of mice with this mixed virus stock, which contains a 4.9kb replication defective genome (BM5def) and B-tropic replication competent ecotropic and MCF helper MuLVs, results in progressive lymphoproliferative disease and immune system impairment, the disease process requiring BM5def and both B and CD4$^+$ T cells. For studies of the precise role of BM5def in disease induction, cells have been transfected with the molecularly cloned genome, either native or modified by insertion of the marker gene for neomycin resistance (BM5def/neo^r). Analysis of virus rescued by use of replicating ecotropic or amphotropic helpers has shown that 1) the disease induced by helper-rescued BM5def is indistinguishable biologically and molecularly from MAIDS induced by LP-BM5 mixture; 2) MCF virus enhances efficiency of disease induction but is not required; 3) virus stocks rescued by cells cotransfected with neo^r and BM5def or transfected with BM5def/neo^r induce MAIDS but the cells and tissues from infected mice often contain BM5def-hybridizing fragments of sizes that reveal mutations in restriction endonuclease sites. In extending studies of whether MAIDS predisposes mice to secondary disease induced by normally innocuous agents, we have shown that 1) the severity of M-CMV-induced inclusion body disease is increased and extended in duration in adult mice infected for at least 6 wk with LP-BM5 MuLV; and 2) natural or experimental infection with C. freundii can result in hyperplastic to ulcerative colitis, often fatal. In studying the dysfunction of both CD4$^+$and CD8$^+$ T cells in mice with MAIDS it was shown that in infected mice impaired responses to mitogenic and antigenic stimuli were correlated with impaired calcium influx responses and the frequency of lymphocytes with prestimulation intracellular calcium was elevated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00286-11 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Janet W. Hartley Head, Viral Oncology Section, LIP, NIAID
Others:	H.C. Morse III, Chief, LIP, NIAID
	T.N. Fredrickson, Expert, NCI
	Y. Tang, Visiting Associate, LIP, NIAID
	T. Torrey, Senior Staff Fellow, LIP, NIAID

COOPERATING UNITS *(if any)*

M. Makino (Natl. Inst. of Health, Japan), R. Schwartz, NIAID, D. Murphy (NY State Dept. of Health); I. Egorov (Jackson Labs), M. Zijlstra (Whitehead Inst), R. Melvold (Northwestern Univ Med School).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.8	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The present emphasis of this project is the study of host genetic determinants of sensitivity and resistance to induction of MAIDS by the replication defective virus termed BM5def MuLV. Differences in susceptibility to MAIDS among inbred strains of mice reflect the function of host genes, both within and outside the MHC complex, that may influence infection by and spread of the replication competent viruses that act as helper for spread of the defective virus genome or may act in primary control of the spread or activity of BM5def. Using LP-BM5 MuLV, which contains B-tropic helper MuLVs, for Fv-1^b strains, mice of the H-2 haplotypes b, j, k, p, r and s were found to be moderately to highly sensitive while H-2^d and H-2^a strains were resistant. By testing mice with recombinant MHC complexes, resistance was mapped in association with class I molecules, and in some strains the d haplotype at the D-end of H-2 was implicated. In further tests of MHC Class I effects we have found that mutations at H-2D can render a resistant mouse sensitive, and mice of a resistant strain inoculated with LP-BM5 MuLV and then depleted of CD8$^+$ T cells developed MAIDS and integration of the defective genome was demonstrated. In some resistant strains but not all, helper virus replication is suppressed, probably by effects of both MHC and non-MHC genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00465-07LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retrovirus-induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, Chief LIP, NIAID
Others: Janet W. Hartley Head, Viral Oncology Sect. LIP, NIAID
Sisir Chattopadhyay, Expert, LIP, NIAID
Ambros Hügin, Guest Researcher, LIP, NIAID
Nathalia Giese, Visiting Fellow, LIP, NIAID
A.S. Rosenberg, Senior Investigator CBER DV, FDA
T.N. Fredrickson, Expert NCI
R.T. Gazzinelli, Staff Fellow LPD, NIAID

COOPERATING UNITS *(if any)*

Hospital Cantonal Universitaire, Geneva, Switzerland (A. Cerny, F. Waldvogel, S. Izui), Hopkins Cancer Center (P. Pitha) Washington University School of Medicine (O. Kanagawa), National Institute of Health, Tokyo, Japan (M. Makino), Dartmouth

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at developing an in-depth understanding of how retroviruses induce immunodeficiency and how this disorder can be prevented and treated. Studies are based on analyses of mice infected with a unique set of retroviruses, designated LP-BM5 murine leukemia viruses (MuLV), that develop a disease, termed mouse AIDS (MAIDS), with many similarities to HIV-induced disease in humans. The etiologic virus in this mixture is a defective MuLV (BM5def) which encodes a gag protein that appears to act as a superantigen. Mice infected with the viruses develop both humoral and cellular immune responses to BMdef. In A/J mice, a strain resistant to development of MAIDS, an anti-BM5def response by $CD8^+$ T cells effects clearance of the virus, thus preventing disease. $CD8^+$ CTL are also responsible for restricting the spread of ecotropic helper virus in mice of this strain. $CD8^+$ responses to BM5def-encoded antigens can also be induced in MAIDS-sensitive mice, but appear to be inadequate to control virus spread. In other studies, cyclosporin A, a drug that limits $CD4^+$ T cell cytokine production was active in preventing rapid progression of MAIDS. By combining cyclosporin with the anti-viral drug ziduvodine (AZT), an additive effect could be seen on lymphoproliferation. The large proportion of $CD4^+$ cells proliferating in mice sensitive to MAIDS can be ascribed to the high frequencies of BM5def-responsive cells found among T cells expressing Vβ5.1, 5.2, 8.2, 11, 12, 14 and 17a. Using mice bearing some of these T cell receptor specificities as transgenes, it will be possible to have large numbers of cells responsive to the MAIDS superantigen for analyses of the biochemical events responsible for the state of anergy that characterizes the late stages of MAIDS. In mice with long term disease, splenic T cells are nonresponsive, as measured by proliferation, to stimulation through the T cell receptor or through CD28. They also fail to respond following stimulation by PMA and ionomycin. This contrasts with a relative conservation of function by lymph node cells from the same mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00484-06 LIP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Kevin L. Holmes Head, Flow Cytometry Section, BRB, NIAID
Others: H.C. Morse III, Chief, LIP, NIAID
L. Staudt, Investigator, DCBD, NCI
P. Sherle, IRTA Fellow, DCBD, NCI
J. Taubenberger, Expert, Staff Surgical Pathologist, LP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Immunopathology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of this project is to promote a clearer understanding of the mechanisms of hematopoietic cell differentiation by characterizing cell surface antigens expressed during the development and after the maturation, of the blood elements. We have primarily focused on the characterization of a novel antigen identified by a monoclonal antibody LIP-6. LIP-6 was produced against an undifferentiated murine hematopoietic cell line in order to identify antigens expressed early in development. LIP-6 is expressed by bone marrow cells, B lineage cells and macrophages but absent on all T lineage cells. Experiments have shown that antibodies to LIP-6 can induce proliferation of mature B cells in the absence of T cells, suggesting that LIP-6 may be important in activation of mature B cells. In addition, a population of bone marrow cells has been identified by its expression of LIP-6. Characterization of this LIP-6 precursor population by combined genetic and biological assays suggest this population is an immature progenitor population with limited differentiation capacity within the B cell lineage and perhaps the myeloid lineage. Future studies will further characterize the LIP-6 antigen and the population of cells expressing LIP-6 in bone marrow. These studies will hopefully yield new information about the cellular interactions important in B development and B lymphocyte interactions.

Two other cell surface antigens expressed on bone marrow, BRB44 and Ly-26.1 are also being studied. BRB44, produced in our laboratory against an undifferentiated cell line, has a very complex strain distribution pattern among inbred strains of mice and is expressed on mature B and myeloid lineage cells. Activation of B cells, as occurs in mice with MAIDS, induces the expression of BRB44 on B cells and $CD4^+$ cells in B6, $BRB44^{lo}$ mice, suggesting that BRB44 is an activation antigen. Recent characterization of a previously described antigen, Ly-26.1, has shown that it is variably expressed on most bone marrow cells, mature B lymphocytes and granulocytes/macrophages and on $CD4^+$ T cells. Interestingly, the binding of antibodies to the Mac-1(CD11b) surface antigen is blocked by anti-Ly26.1 antibodies. These data and preliminary biochemical data suggest that Ly-26.1 may be co-associate with Mac-1 on the surface of myeloid lineage cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00544-04 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis of Pathogenesis of Murine Leukemia Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Sisir K. Chattopadhyay, Expert, LIP, NIAID
Others: H.C. Morse III, Chief, LIP, NIAID
J.W. Hartley, Head, Viral Oncology Section, LIP, NIAID
Torgny N. Fredrickson, Expert, NCI
Yao Tang, Visiting Associate, LIP, NIAID
Ambros Hügin, Visiting Associate, LIP, NIAID
Wan Kee Kim, Visiting Fellow, LIP, NIAID

COOPERATING UNITS *(if any)*

Veterans Administration, Baltimore, MD (J. Eiseman, J. Bilello)
Yale University (Richard Flavell)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A combination of biological and molecular techniques permitted us to identify that a 4.9kb replication defective(def) recombinant murine leukemia virus, present in the LPBM5 murine leukemia virus complex, as the proximal cause of a syndrome, murine AIDS (MAIDS), manifested by lymphoproliferation and immunodeficiency. Molecular cloning and sequence analysis revealed that the defective virus has a functional LTR and the *gag* gene is intact. The other two genes i.e *pol* and *env* are severely deleted. Within the *gag* gene p10 and p30 are very closely related to replication competent helper ecotropic virus whereas the p15 and p12 are most divergent. A 100bp probe was developed from the p12 region and used to follow the infection in animals. The presence of defective genome could be demonstrated 2 wk after infection in sensitive C57BL/6 mice, whereas such genomes were absent from the tissues of resistant A/J mice even after 12 wk of infection. Late stage MAIDS is regularly associated with clonal expansion of B cells. Using a potentially more sensitive assay for the presence of transformed cells by transferring cells from MAIDS mice to mice with *scid* mutation, we have been able to show that a) transferrable tumors are present in tissues of mice beginning 12 wk post infection; b) tumor types present include CD4$^+$ T cell as well as pre-B and B cell lymphomas; c) both T and B lineage lymphomas may occur in a single mouse; d) the tumors are clonal for J_H or T cell receptor gene rearrangements and e) most if not all tumors have clonal integrations of the BM5 def genome. Bacterially expressed p12 *gag* induced high levels of antibodies in both susceptible and resistant mice but failed to induce a protective immune response. We developed a pathogenic defective virus containing a bacterial neomycin resistant gene which will facilitate quantification of the virus, *in situ* hybridization study to determine the initial target cell(s) and further generation of mutant viruses to determine the role of various *gag* proteins. Transgenic mice containing the entire *gag* gene of the def. virus under the control of SV40 promoter and immunoglobulin heavy chain enhancer were developed. Such mice may prove useful in understanding the role of *gag* in disease development.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00578-03 LIP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Oncogenes in B Cell Neoplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Shapiro, Staff Fellow, LIP, NIAID
Others: H.C. Morse III, Chief, LIP, NIAID
J.F. Mushinski, Investigator, LG, NCI
C.A. Kozak, Research Microbiologist, LMM, NIAID
E.A. Campbell, IRTA Fellow, LIP, NIAID
C. Jhappan, Staff Fellow, LMB, NCI

COOPERATING UNITS *(if any)*

Institute of Medical and Veterinary Science, Adelaide, South Australia (W. Langdon)

LAB/BRANCH

Laboratory of Immunopathology, NIAID, NIH

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of neoplasms in vertebrates is often associated with alterations in the structure or expression of cellular proto-oncogenes. We have been characterizing the cbl proto-oncogene which was first discovered in a defecteve retrovirus that induces pre-B lymphomas in vivo. The sequences of 4 cDNA clones (2 human and 2 mouse) suggest that cbl may be a transcription factor. There is homology with the GCN4 transcriptional activation region, a proline rich region, a basic region and a leucine zipper. One of the murine cDNAs, however, differs completely at its 3' end in that it is lacking the leucine zipper motif and would encode a shorter protein. There are no nucleotide differences between v-cbl and c-cbl, rather, v-cbl is a truncated form of the gene which also does not contain the leucine zipper. Sequencing of the germline locus has identified 7 exons so far, ranging in size from 120 to 201bp. Cbl is not an immediate early gene and its expression is not regulated upon the addiiton of mitogens to various cell types. Cbl, however may play a role in the differentiation of certain cell types since terminal differentiation of an erythroid and of a promyelocytic cell line shuts off cbl expression. NIH3T3 cells have been transfected with various cbl constructs. A construct with c-cbl is weakly transforming while a construct with v-cbl is only slightly better at transforming these cells. The construct containing the c-cbl lacking the leucine zipper did not transform cells at all. Transgenic mice have been developed containing constructs with either v-cbl or c-cbl in conjunciton with the IgH enhancer. To date, the c-cbl transgenic mice have not developed tumors but spleens from two mice did not show elevated levels of cbl expression. Transgenic mice containing v-cbl are in the process of being bred for further studies.

LABORATORY OF IMMUNOREGULATION
1992 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1991 through September 30, 1992

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation

Immunopathogenic Mechanisms of HIV Infection

Over the past several years the LIR has been involved in studying the immunopathogenic mechanisms of human immunodeficiency virus (HIV) infection with particular attention to the modulation of HIV expression from the latent to the replicative state by normal endogenous immunoregulatory cytokines. Most recently we have focused on the role of lymphoid organs in the pathogenesis of HIV infection with regard to their role as reservoirs of HIV and predominant sites of HIV replication. With regard to the former, we have established that a panel of cytokines involved in normal immunologic homeostasis are capable of modulating HIV expression in a paracrine and autocrine fashion. Specifically, tumor necrosis factor-alpha and beta (TNF-α and β) upregulate HIV expression via activation of the cellular transcription factor NF-κB whereas other cytokines such as interleukin (IL)-6 and granulocyte-macrophage colony stimulating factor (GM-CSF) induce virus expression predominantly by post-transcriptional mechanisms. Marked synergy among these factors can be demonstrated indicating that *in vivo* the complex immunoregulatory network of cytokines likely plays an important role in the maintenance of HIV expression. Transforming growth factor (TGF)-β has bifunctional effects on HIV expression depending on the experimental conditions. It blocks both transcriptional and post-transcriptional induction of HIV from chronically infected cells by phorbol-12-myristate 13-acetate (PMA) and IL-6/GM-CSF as well as by glucocorticoids in combination with cytokines. On the other hand it induces HIV expression in acutely infected cell lines and primary monocyte-derived macrophages. Most recently we demonstrated that interferon (IFN)-γ induces expression of HIV either alone or in synergy with TNF-α. Although it appeared to block the induction of HIV from the chronically infected promonocytic cell line U1 by PMA, in reality it redirected virion budding from the plasma membrane to the intracytoplasmic vacuoles of the cell concomitant with the differentiation of the cell creating a reservoir of HIV within the cell. This has important physiologic and therapeutic implications.

After having established the role of NF-κB in the induction of HIV transcription by TNF-α, we further established that the SP-1 promotor regions of the HIV long terminal repeat (LTR) are essential for high constitutive expression of HIV while NF-κB binding activity rescues transcription for SP-1 mutants if cells are treated with TNF-α. We also utilized site directed mutagenesis of the NF-κB p50 protein and delineated the distinct regions necessary for protein binding to HIV DNA consensus sequences and for dimerization.

We have extended our studies on the pharmacologic modulation of HIV expression. We have previously demonstrated that glucocorticoids could synergize with TNF-α or IL-6 in the induction of HIV expression while not being inductive alone. We further showed that retinoic acid mimicked TGF-β in its bifunctional effects on HIV expression and that the anti-oxidants glutathione (GTH) and N-acetyl-cysteine (NAC) blocked both PMA and cytokine induction of HIV expression.

Over the past year we demonstrated that glucocorticoids synergize with TNF-α by a transcriptional mechanism and that NAC, unlike GTH, suppressed HIV transcription in U1 cells and that this was strongly correlated with NF-κB activation. Furthermore, we demonstrated that the platelet activating factor (PAF) antagonist RP55778 blocked expression of HIV in U1 cells following induction by PMA, IL-6 or GM-CSF. The molecular mechanisms were complex involving both transcriptional and post-transcriptional events. It post-transcriptionally blocked HIV expression by all 3 stimuli, but blocked the PMA induction of TNF-α transcription in U1 cells thereby interfering with the PMA-induced autocrine pathway of TNF-α.

Major progress was made in the delineation of the role of lymphoid organs in the immunopathogenesis of HIV infection over the past year. Considerable confusion exists around the concept of clinical latency versus microbiological latency in HIV-infected individuals. Following primary infection and the burst of viremia that ensues, most patients enter a clinically latent state in which few (1:10,000 to 1:100,000) cells in the peripheral blood express HIV and viremia is low or absent. Patients are generally asymptomatic, but immune deterioration inexorably progresses until clinically apparent disease results. We simultaneously studied peripheral blood and lymph node tissue in the same HIV-infected individuals utilizing DNA polymerase chain reaction (PCR) to determine viral burden and RNA PCR to determine HIV expression. In addition, we examined the lymph nodes for the state of its architecture and for number and location of infected cells by *in situ* hybridization. We demonstrated that at any given time during the course of infection from the early asymptomatic stage through the stage of AIDS, the viral burden in the lymph node was 1 to 2 logs greater than that in the peripheral blood. Furthermore, substantial virus expression was consistently demonstrated in the lymph node early in the course of infection when this was at low levels or absent in peripheral blood cells. Histopathologic and *in situ* studies revealed that early in the course of infection antibody coated virions are trapped in the follicular dendritic cells (FDC) of the germinal centers exposing the adjacent cells to activation and infection. However, the primary cells infected in the lymph node early in the course of infection are the paracortical $CD4^+$ T cells. Late in the course of infection the FDC are infected and disrupted leading to loss of germinal center architecture and the appearance of a "burnt out" node. We feel that this stage coincides with the accelerated viremia noted late in the disease which is likely due at least in part to a failure of the trapping mechanisms of the FDC. Hence, active replication of HIV occurs throughout the course of HIV infection and a true latent state does not exist in HIV infection. HIV replication and disease clearly progresses during the clinically latent state.

Recent studies in other laboratories have suggested that HIV-encoded proteins or other microbial components may serve as superantigens in HIV infected individuals. Unlike conventional antigens which trigger a very small fraction (less than 1%) of T cells, superantigens are capable of triggering large proportions of T cells which bear certain Vβ families of their T cell receptor (TCR). In animal models, superantigens are responsible for the activation, expansion and ultimate deletion of selective Vβ subsets of T cells. It is hypothesized that the dysfunction and/or deletion of $CD4^+$ T cells in HIV-infected individuals might be explained in part by exposure to a superantigen(s). We have examined the Vβ repertoire (24 vβ families) of pairs of identical twins discordant for HIV infection and have demonstrated perturbations of certain Vβ subsets in the infected twin. Twin studies were essential for this observation because of the strict dependence of the Vβ usage on major histocompatibility complex. These preliminary studies suggest that a superantigen is operative in HIV infected individuals and may play a role in immunopathogenesis.

It is an established fact that the clinical course in HIV-infected infants is more fulminant than that in adults. In attempting to identify the factors which may contribute to this phenomenon, we examined the plasma from large numbers of infants born of HIV-uninfected and HIV-infected mothers in the USA and in Zaire. Markedly elevated levels of TNF-α and IL-6 were demonstrated during the first week of life in all infants likely representing a normal physiologic response to environmental stimuli. Since these cytokines are capable of inducing HIV expression (see above), these findings may help explain the accelerated course of HIV disease observed in certain infants.

We have extended our studies on the mechanisms of cytopathicity of HIV-infected cells and have demonstrated that HIV-mediated syncytia formation in $CD4^+$ T cells is dependent on the LFA-1/ICAM 1,2,3 pathway of cell to cell adhesion.

In order to dissect the molecular mechanisms by which the HIV kills $CD4^+$ T cells, we have engineered $CD4^+$ T cell lines expressing individual or groups of HIV-1 genes, and analyzed signalling abnormalities in HIV-infected cells. T cell lines transfected to express the envelope glycoproteins grew normally. The co-culture of envelope-expressing cells with naive $CD4^+$ cells led to syncytium formation, cell death, and alterations in intracellular signalling pathways as assessed by the tyrosine phosphorylation of intracellular substrates. Blockade of these tyrosine phosphorylations with the protein tyrosine kinase inhibitor, herbimycin A, dramatically reduced cytopathicity in HIV-infected cultures and in co-cultures between HIV-envelope expressing cells and $CD4^+$ cells. The regulatory tyrosine phosphorylation of a substrate which we have determined to be the cyclin dependent kinase cdc2 was most closely associated with syncytium formation and cell death. Numerous studies have shown that the cdc2 protein has a central role in controlling transitions in the cell division cycle, and its disregulation in HIV infection could be contributing to cell death.

In work designed to initiate adoptive immunotherapy for HIV, we have cloned, sequenced and expressed the antibody genes isolated from a human anti-HIV gp41 monoclonal cell line. These antibody genes were genetically modified to donate their variable region combining sites onto TCR constant regions, generating a chimeric antibody/TCR which was expressed on T cell lines. (Poli, Pantaleo, Stanley, Bressler, Graziosi, Biswas, Weissman, Brown, Butini, Vaccarezza, Masci, O. Cohen, Lane, D. Cohen, Tani, Siebenlist, Fauci, LIR/NIAID; Orenstein, GWU; Fox, Yale; Kotler, St. Luke's Hosp., NYC; Springer, de Fougerolles, Harvard; Haynes, Duke; Kessler, NMRI; Sekaly, Univ. of Montreal; Shaw, Saag, Univ. Alabama).

Regulation of Gene Expression of the Human Immunodeficiency Virus

Recently we have proposed a model for the mechanism of action of the *Rev* protein of HIV-1 which holds that *Rev* cooperatively binds with cellular factors and the *Rev*-response element (RRE) to form a functional complex. Using cooperative binding as a criteria to search for *Rev* accessory factors we have detected in crude extracts a nuclear activity which promotes the binding of *Rev* to the RRE. This activity either cooperatively binds to the RRE with *Rev* or catalyzes the binding of *Rev* to the RRE. Almost certainly the activity is protein. Chromatographic analysis of the extracts has resolved at least three and possibly as many as five separate activities. Currently we are purifying the separate activities and cloning them. In parallel with these studies, we have detected the existence of cell-type specific cellular factors by studying the replication kinetics in different cell types of HIV proviruses with RRE mutations. In different cell types, HIV proviruses with different RRE mutations replicate with drastically different kinetics. This data is the first reported indication that the RRE may contribute to cell-type specific viral tropism. In related work we have studied the interaction of the *Rev* protein with the RRE. We have found that RRE determinants strongly influence the binding of *Rev* molecules subsequent to the first binding event. This precludes models for *Rev*/RRE binding which hold that all of the specificity is in the first binding event and that subsequent multimerization of *Rev* along an RNA molecule involves only non-specific recognition of RNA by *Rev* protein. This work has also identified RRE molecules that tightly bind *Rev* but that do not permit more than two *rev* molecules to bind. Functional analysis of theses mutations will assess the requirement of multimerization for function. (A. Dayton, E. Dayton, Powell, Bende, Lee, Butini, Pantaleo, Fauci, LIR/NIAID; Maizel, Shapiro, LMB/NCI).

Clinical Investigation of the Acquired Immunodeficiency Syndrome

An intensive effort to develop better treatment for patients with HIV-1 infection continued . A series of clincal trials directed toward therapy of HIV-1, its complications and immunorestoration were conducted over the past year. These trials were, for the most part, phase I/II in design and in multiple instances provided important information regarding the potential benefit of individual approaches to therapy.

In the area of anti-retroviral therapy, three new trials were initiated. A phase II study was conducted with the non-nucleoside reverse transcriptase inhibitor L697,661. This study demonstrated, in the context of a placebo and zidovudine controlled trial, that plasma viremia can be a sensitive marker for *in vivo* anti-retroviral activity. A phase I trial of the immunotoxin CD4-PE was initiated and is currently ongoing. This trial is designed to test the concept that therapy with an immunotoxin directed toward HIV-1 infected cells is capable of decreasing overall viral burden. Similarly a phase I study of NAC is attempting to determine whether or not increasing intracellular glutathione levels, and thus decreasing the rate of HIV-1 LTR transcription, is capable of modifying the *in vivo* replication of HIV-1. Thus far these trials have not demonstrated significant *in vivo* effects for either of these two agents.

Work this year in the area of AIDS-related opportunistic infections has focused on *Pneumocystis carinii*, cytomegalovirus (CMV) and cryptosporidiosis. We have demonstrated in a phase I/II trial that the hydroxynapthaquinone BW566C80 is an effective agent for the treatment of *Pneumocystis carinii* pneumonia and that this efficacy is just slightly less than that of standard treatment. In addition, this agent was demonstrated to have the potential for wide-reaching clinical use by virtue of being effective in the treatment of patients with cerebral toxoplasmosis refractory to conventional therapy. A delayed therapy controlled trial of patients with CMV retinitis with foscarnet showed clear-cut benefit for immediate therapy and this study formed a major basis for the FDA approval of this agent for treatment of CMV disease. Work in the area of CMV infection is now focused on the role of CMV-IgG in prophylaxis.

Evaluation of the role of immunologic reconstitution continues as an important goal of the LIR. In the past year we have demonstrated that syngeneic bone marrow transplantation utilizing donors immunized to the HIV-1 envelope protein gp160 caused transient improvements, at best, in immune function. We have initiated studies of the role of a nef-specific cytotoxic T cell clone in treatment with a series of infusions of the clone into the patient from whom it was derived. This trial continues with intensive virologic monitoring. To expand this general area, work has begun on creating a series of HIV-1 human monoclonal antibodies by creating recombinatorial libraries and by establishing the SCID-Hu mouse as a system for the pre-clincal testing of immune based therapies. (Polis, Kovacs, Davey, Walker, Falloon, Fauci, Lane, LIR/NIAID; Masur, CC/NIH; Nussenblatt, NEI/NIH)

International Studies on the Acquired Immunodeficiency Syndrome

A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In one study in Port-au-Prince, Haiti, we demonstrated that nearly 10% of pregnant women are currently HIV-infected with a 2% annual seroincidence. In a separate study in Kinshasa, Zaire, we demonstrated a 40% infection rate in female prostitutes with an estimated 10% annual seroincidence. Among patients attending sexually transmitted diseases (STD) clinics in Baltimore, a retrospective 10-year survey demonstrated a marked increase in HIV infection from 0.2% to 5.5%, with a current annual seroincidence of 2.0%. Common to all three cohorts was the strong association of HIV infection among heterosexuals with past or active genital ulcerative and non-ulcerative STDs, smoking, lack of circumcision, and inconsistent use of condoms. In following over 500 children born to HIV-infected women, we have documented that 28% of the children acquire HIV infection perinatally, which is independently associated with depressed maternal CD4$^+$ lymphocyte counts, anemia, chorioamnionitis and funisitis. We were unable to document any association between the presence or absence of neutralizing antibodies to the V3 loop and perinatal transmission. Following acid association of serum immune complexes, we were able to demonstrate extremely high levels of p24 antigenemia during the first 3 months of life in perinatally infected children. When used in combination with either PCR or an IgA assay for HIV-specific antibodies recently developed in our laboratory, the diagnosis of perinatal HIV infection could be established within the first 3 months of life with a sensitivity and specificity of 99%, respectively. (Quinn, Brown, Fauci, LIR/NIAID; Hook, Rompalo, Zenilman, Halsey, Kelen, Johns Hopkins).

Isolation and Characterization of Genes Important in B Cell Function

A number of different approaches have been used to isolate genes important in B cell function and lymphocyte activation. Using subtractive hybridization we isolated a series of cDNA clones which are differentially expressed between B and T lymphocytes. Several of these genes have been further characterized. One of these genes encoded for the B cell membrane protein CD22. The structure of the CD22 gene has been determined, its chromosomal location found, and the transcription start sites mapped. Two other subtracted clones, BL11 and BL34, have been characterized. BL11 mRNA is expressed in activated B cells and was found to encode a membrane protein belonging to the immunoglobulin super family. BL34 mRNA is also expressed in activated B cells and was found to be highly expressed in B cell acute lymphocytic leukemia. A novel protein kinase, GCK, has been found to be differentially expressed between mantle zone and germinal center B cells. In an attempt to understand the mechanisms of B cell specific gene expression the promoter regions of several B cell specific genes, CD19, CD20, and CD22, are being studied. The most progress has been made with the CD20 promoter. Several sites within the promoter have been identified which are important in the regulation of CD20 gene expression.

One of the sites was found to be a diverged octamer binding site. Oct-2 was found to be important in the B cell specific expression of CD20 in mature B cells and to be important in the induction of CD20 expression in a pre-B cell line. *In vivo* footprinting is being used to characterize the promoter regions of CD20 as well as the other promoters. We have generated 5 CD19 negative B cell lines by mutagenesis of a CD19 positive cell line. These cell lines should prove useful in the analysis of the CD19 promoter. These studies should provide a basis for understanding the B cell restricted pattern of expression of these genes.

The locations of the HB9 and HB24 genes map to the long arm of chromosome 1. *In situ* hybridization could not distinguish between the two loci. The human HB24 gene was introduced into the genome of a mouse under the control of the murine T cell receptor promoter/enhancer. The resultant HB24 transgenic animals had some interesting phenotypic abnormalities including elevated numbers of activated T cells although largely in the CD4 subset, impairment of $CD4^+$ T cell development, and a failure of the thymus to undergo normal age related involution. The exact role of HB24 in these abnormalities is yet to be determined. Both HB9 and HB24 are known to be expressed at high levels in hematopoietic progenitors. We have provided evidence that HB24 is involved in the proliferation of hematopoietic progenitors and that differentiation of hematopoietic progenitors along specific cell lineages may require downregulation of HB24 expression. Both HB9 and HB24 were found to be expressed at high levels in acute myelogenous leukemic cells as well as acute lymphocytic leukemic cells. In contrast, normal levels of HB24 and HB9 were found in RNA from bone marrow and peripheral blood B cells from patients with chronic leukemias. The levels of HB9 and HB24 were found to correlate with the clincal status of the patients with acute leukemias. A human T cell line transfected with the HB24 gene was found to induce tumors when injected into nude mice, in contrast, control cell lines failed to induce tumors. The tumors were clearly of human origin and maintained the phenotype of the transfected cell line. These studies suggest the possibility that over-expression of HB24 may have an important role in leukemogenesis. (Kehrl, Deguchi, Hong, Kozlow, Thevenin, Riva, Fauci, LIR/NIAID; Colligan, BRB/NIAID; Kirschenbaum, NIAID; Najfeld, Mayer, Mt. Sinai, NY; Katz, Georgetown; Fox, Yale).

The Genetic Response of Primary T Lymphocytes to an Activation Signal: The Role of NF-κB and Other Genes in the Control of Immune Competence and Proliferation

We have cloned and characterized a number of mitogen-inducible cDNAs isolated from our subtractive T cell library. Included among the encoded products of these genes are several cytokines, a transmembrane protein, a ras-related protein, a phosphatase, a steroid-receptor-like protein and several different transcription factors. Given their similarity with known proteins these rapidly induced gene products are likely pleiotropic effectors of the initial activation signal. Several of these proteins also resemble known signal-transducing proteins, allowing for further controls of the induced proteins by additional signals. In this scenario multiple decision points precede commitment to proliferate.

A primary focus of our laboratory has been on the NF-κB transcription factor. This transcription factor is uniquely positioned during activation to play a major role in establishing immune competence: A large number of immunomodulatory factors and receptors are regulated by it. In addition, the HIV is highly responsive to NF-κB. We have for the first time cloned via our subtractive approach two genes encoding NF-κB activity, p50 and p50B. We determined that

NF-κB is a family of homo- and heterodimeric complexes. The predominant form of NF-κB is a p50/p65 heterodimer which is bound to its repressor IκB in the cytoplasm of cells. The preformed complex is primed for a signal which releases the inhibitor and thus allows for nuclear entry and DNA binding. In addition to p50/p65 other complexes exist, including those involving p50B, a protein unknown prior to cloning. p50B forms heterodimers with p65, c-Rel or RelB, proteins with known transactivation domains. p50B also synergizes with these proteins in transactivating κB-dependent promoters. We demonstrated with specific antibodies that both p50 and p50B are processed from activation-induced precursor molecules. These proteins are present in preformed complexes in resting cells but are also induced upon activation. This implies that NF-κB activity in cells comes in two distinct forms, one which depends on preformed complexes and another which depends on new synthesis following activation.

Through the use of specific antibodies we discovered a large pool of p50 homodimers in the nucleus of resting cells. These p50 homodimers could act as competitive repressors of transactivating complexes, suggesting that they play a repressing role. These repressing homodimers in turn appear to be the target of another mitogen-inducible gene product, the Bcl-3 oncogene, which is structurally related to IκB. Bcl-3, by physically associating with p50, could counteract p50 homodimers, allowing κB-dependent transactivation by heterodimers like p50/p65. Our work shows that NF-κB activity in cells is defined by a complex network of positively and negatively acting components. Such complexity in the components is likely to be reflected in multiple regulatory phenomena governing κB-dependent gene expression. (Siebenlist, Bours, Brown, Franzoso, Kanno, Bressler, Fauci, LIR/NIAID; Kelly, LP/NCI; Bravo, Squibb Inst. Mol. Biol.)

Clinical, Immunopathogenic, and Therapeutic Studies in the Vasculitides and Other Immune-Mediated Diseases

Our protocols of cyclophosphamide (CP) and alternate day glucocorticoids (GC) for the treatment of Wegener granulomatosis (WG) and other systemic vasculitides have been extremely successful in inducing remissions in these formerly fatal diseases over the past 26 years. However, because of the long term toxicity observed with CP and the relatively high relapse rate (40-50%) over a prolonged period of time, we have been investigating the use of alternative therapies in these diseases.

We have studied treatment with weekly low dose methotrexate (MTX) and GC in patients with WG and Takayasu arteritis. Fifty-two patients with WG have received MTX, including 29 with at least 6 months followup. Sixty-one percent of WG patients have achieved a MTX-induced remission and 64% have so far successfully discontinued GC therapy. Fifteen patients with Takayasu arteritis have also been treated with MTX and GC. These patients had previously either been GC-unresponsive or unable to achieve complete remission with GC therapy. In seven instances, the addition of a cytotoxic agent (CP in 6 cases, azathioprine in 1 case) also failed to provide GC-free remissions. Eight of 15 Takayasu arteritis patients thus far treated with MTX have achieved GC-free remissions and 5 additional patients who are in the process of tapering GC are taking 50% less GC than had previously been possible. Apart from two patients having to discontinue MTX because of presumed drug-induced pneumonitis, this agent has been relatively well tolerated. The ultimate role of MTX in the treatment of these diseases is uncertain because of the small number of patients studied thus far and the short period of follow-up (mean period for WG = 15 months, and for Takayasu arteritis = 30 months). Patients with these diagnoses and other forms of systemic vasculitis will continue to be enrolled in each of these studies.

Our studies of children with WG have demonstrated that children have a much greater incidence (48% vs 10%) of subglottic stenosis and nasal destruction (48% vs. 25%) than adults. The reasons for this distinction are unclear, but may relate to susceptibility of cartilaginous structures during periods of more rapid growth. Morbidity from disease and periods of more rapid growth. Morbidity from disease and treatment was substantial in children and encourages additional studies of alternative therapies. Treatment of systemic vasculitis would be greatly facilitated by a reliable laboratory marker of active disease. It has been suggested by others that anti-neutrophil cytoplasmic antibody titers represent such a tool in WG. However, our studies of serial titers in 53 patients indicate that this test is not adequately sensitive to be used as a sole criterion to modify treatment. (Hoffman, Leavitt, Kerr, Cid, Fauci, LIR/NIAID; Rottem, LCI/NIAID; Kleinman, Grant, LDBA/NIH; Hallahan, NIAID; Cotch, Kaslow, DMID/NIAID; Dickler, DAIT/NIAID; Lebovics, DIR/NIDCD).

Pathogenesis of *Chlamydia trachomatis* Infections

Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop improved molecular diagnostic assays and to examine the pathogenesis of chlamydial infections in experimental animal models and in patients with genital, ocular and respiratory infections. Utilizing a variety of diagnostic assays, chlamydial genital infections continue to be highly prevalent among patients attending STD clinics with rates of 10 to 15%. Utilizing PCR, we have been able to demonstrate a marked increase in sensitivity compared to culture in identifying patients with chlamydial genital infection. PCR was positive in 75% of male partners to infected females as compared to 39% by culture. Sequence analysis of PCR amplified DNA demonstrated the same sequence in paired sexual partners illustrating the accuracy and validity of PCR. Utilizing urine specimens from asymptomatic men, we identified chlamydial infection by PCR in 11% of 1,000 asymptomatic men similar to a 13% infection rate among 500 symptomatic men. We demonstrated that PCR could also be used to identify chlamydial infections in children with trachoma and to monitor response to therapy. With PCR amplification, it was evident that nearly 50% of treated children had a relapse as early as 4 weeks post-therapy. Persistence of chlamydial DNA and RNA was demonstrated by PCR in monkeys who had become culture negative. This persistence of chlamydial DNA appears to contribute to a sustained hypersensitivity response related to the secretion of gamma interferon by the host and heat shock protein (HSP-60) by the organism. Following the sequencing of the 16s rRNA of *C. pneumoniae*, specific primers were identified and utilized for PCR detection of *C. pneumoniae* in patients with respiratory disease. In over 500 evaluated patients, PCR detected *C. pneumoniae* in 4% of patients and in none of asymptomatic healthy controls. Additional studies utilizing PCR are planned to further describe the clinical features and transmission of both *C. trachomatis* and *C. pneumoniae* in selected populations at risk. (Quinn, LIR/NIAID; Hook, Bobo, Viscidi, Johns Hopkins)

Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR have received a number of awards and honors. Dr. Fauci continues to serve on a number of committees of scientific and administrative importance such as the Institute of Medicine Roundtable for the Development of Drugs and Vaccines against AIDS. He is chairman of the NIH AIDS Executive Task Force as well as a member of the U.S. Public Health Service AIDS Task Force and the PHS AIDS Executive Committee. He is co-chairman of the subgroups on Therapeutic Intervention and on Vaccine of the PHS AIDS Executive Committee.

This past year, Dr. Fauci continued his role as an advisory editor of *The Journal of Experimental Medicine* and on the Editorial Board of *The Journal of Immunopharmacology, Clinics in Immunology and Allergy, EOS, Cellular Immunology, The Journal of Molecular and Cellular Immunology, AIDS Research and Human Retroviruses,* and *AIDS Patient Care*. He serves on the Advisory Board of *Immunopharmacology and Immunotoxicology, Clinical Immunology and Immunopathology,* and the *Journal of Clinical Immunology*. He is on the Editorial Advisory Council of *La Ricerca in Clinica e in Laboratorio*. He also continued his duties as associate editor of the *Journal of the Acquired Immunodeficiency Syndrome*.

This past year, Dr. Fauci became an associate editor of "AIDS, Etiology, Diagnosis, Treatment, and Prevention." He continues as an editor of "Harrison's Principles of Internal Medicine," "Transactions of the Association of American Physicians," "Current Therapy in Allergy, Immunology and Rheumatology," and "Harrison's Principles of Internal Medicine - Companion Handbook." He is an associate editor of "Current Therapy in Internal Medicine." In addition, he is an editor along with Dr. John I. Gallin of "Advances in Host Defense Mechanisms."

Dr. Quinn continued to serve as President of the American Venereal Disease Association. He was promoted to Professor of Medicine at The Johns Hopkins School of Medicine with adjunct appointments in the Departments of International Health and Immunology and Infectious Diseases at The Johns Hopkins School of Hygiene and Public Health. He was a consultant on AIDS to the Pan American Health Organization and to the National Academy of Science Committee on Population and Social Sciences. Dr. Quinn also served as Chairman of the Committee on Immunology and Infectious Diseases for the NIH Workshop on Research on Women's Health. Dr. Hoffman was elected Chairman of the Multicenter Network for Vasculitis Research.

Dr. Fauci delivered several prestigious lectures including the Tadeusz J. Wiktor Memorial Lecture of the Wistar Institute, the Annual AOA Lecture of the Pritzker School of Medicine of The University of Chicago, and the Nieuwland Sesquicentennial Lecture at The University of Notre Dame. He delivered the commencement address at the Arnold and Marie Schwartz College of Pharmacy and Health Sciences of Long Island University. He was the keynote speaker at the NIGMS Minority Programs Symposium and an invited plenary lecturer at the Pharmacy World Congress and the 8th Annual Wallace P. Rowe Symposium on Animal Virology. Dr. Fauci was also an invited symposium speaker at the annual meeting of the American Society for Virology and the Princeton Conference on Drug Development. Finally, he was an invited plenary speaker at the Institute of Medicine annual meeting.

Drs. Lane, Kehrl, Quinn, Leavitt, Hoffman, Pantaleo, Poli, Graziosi, Siebenlist, Dayton, Stanley, Bressler, and Cohen delivered invited and submitted lectures at a number of national and international meetings throughout the year.

This past year, Dr. Fauci was elected to the National Academy of Sciences (USA) and became a foreign member of the Royal Danish Academy of Sciences and Letters.

Dr. Fauci received a Doctor of Sciences, Honoris Causa, degree from Long Island University, Arnold and Marie Schwartz College of Pharmacy and Health Sciences. The Sons of Italy Foundation established the Anthony S. Fauci, M.D. Perpetual Scholarship.

The 1991 Institute for Scientific Information Database Analysis indicated that of the more than 1 million scientists in the world who published in the decade 1981-1990, Dr. Fauci was the 2nd most cited. He received the Award of Courage from the American Foundation for AIDS Research for "extraordinary leadership and personal scientific contributions to AIDS research."

Dr. Bressler received the American Foundation for Clinical Research Henry Christian Award for Excellence in Research. Dr. Hoffman received the NIAID Director's Award and the NIH Director's Award.

Administrative, Organization, and Other Changes

The major theme of the LIR continues to be the delineation of the cellular and molecular mechanisms of the regulation of the human immune response in normal and disease states. The vast majority of the activities of the LIR currently focus on the human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS), particularly the immunopathogenesis of HIV infection. The size of the laboratory in space, slots and resources has been stable over the past years.

The Section of Vasculitis and Other Related Diseases was established this year and Gary Hoffman, M.D. was named Section Head. Gary Hoffman, John Kehrl and Uli Siebenlist were granted tenure.

The Immunopathogenesis Section of the LIR will move to newly built laboratories located in the A wing of Building 10 in July, 1992.

Dr. Oren Cohen completed the first year of his Clinical Associateship and will join the Immunopathogenesis Section for his research activities. Drs. Michael Granberry and Carlos Muro-Cacho will join the LIR in July, 1992. Dr. Yuexia Li will join the LIR in September, 1992. Dr. Christopher Brown returned from Kinshasa, Zaire and will continue his research in the Immunopathogenesis Section. Eric Kozlow returned to the U.S. Air Force in July, 1992. Dr. Hannelore Ehrenreich returned to Germany after completing a 3 year fellowship. Dr. Ljubisa Vitkovic left the LIR to join the extramural program of NINDS and with his departure the neuropathogenesis unit of the LIR was dissolved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00537-05 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathogenic Mechanisms of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	G. Poli	Visiting Scientist	LIR, NIAID
	G. Pantaleo	Visiting Scientist	LIR, NIAID
	S. Stanley	Medical Officer	LIR, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID
	C. Graziosi	Visiting Associate	LIR, NIAID
	P. Biswas	Guest Researcher	LIR, NIAID

COOPERATING UNITS (if any)

LMM, NIAID (M. Martin); LCP, NCI (M. Sporn, A. Roberts); LVD, NIAID (B. Moss); PB, NCI (P. Pizzo); SB, NCI (S. Rosenberg); MHB, NCI (F. Anderson, R. Morgan); Dept. Pathol. GWU (J. Orenstein); PRI, Fredrick, MD (M. Baseler); St. Luke's Hosp., NYC

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
18	12	6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is directed at delineating the cellular and molecular mechanisms of the immunopathogenesis of human immunodeficiency virus (HIV) infection. We have continued to delineate the inductive and suppressive effects of immunoregulatory cytokines on HIV expression. Extending our previous observations on the effects of tumor necrosis factor-α (TNF-α), interleukin-6, transforming growth factor-β and granulocyte macrophage-colony stimulating factor, we have further demonstrated a synergistic induction of NF-κB dependent HIV transcription in chronically infected U1 promonocytic cells with interferon-α (IFN-γ) and TNF-α in association with terminal cell differentiation. IFN-γ appeared to block HIV induction in U1 cells stimulated with PMA, when in reality it actually redirected virion production from the plasma cell membrane into the intracytoplasmic vacuoles creating an intracellular reservoir of HIV. We demonstrated that SP-1 promoter regions are essential for high constitutive expression of HIV while NF-κB binding activity may rescue transcription for SP-1 mutants if cells are treated with TNF-α. Site directed mutagenesis of the NF-κB p50 protein has delineated regions that are necessary for protein binding and dimerization. A platelet activating factor antagonist (RP55778) was shown to block HIV expression in U1 cells stimulated with PMA or multiple cytokines. We demonstrated that lymphoid organs are the major reservoirs for HIV-infected cells and the predominant anatomic sites for virus replication in that substantial virus replication occurs in the lymph nodes even early in the course of infection when HIV replication is low to absent in peripheral blood during clinical latency. HIV-mediated syncytia formation was shown to be dependent on the LFA-1/ICAMS 1,2,3 pathway of cell to cell adhesion. We demonstrated that distinct sets of Vβ families are perturbed in circulating T cells in HIV infected individuals. Human keratinocytes produce TNF-α in response to UV-B irradiation which is capable of inducing HIV expression in chronically HIV infected cells. We have shown that although CD34$^+$ progenitor cells in bone marrow can be infected *in vivo* and *in vitro* with HIV the bone marrow does not appear to be a major reservoir for the virus.

Continued

Others:	D. Weissman	Medical Staff Fellow	LIR, NIAID
	L. Fox	Medical Staff Fellow	LIR, NIAID
	C. Brown	Medical Officer	LIR, NIAID
	H.C. Lane	Clinical Director	LIR, NIAID
	L. Butini	Guest Researcher	LIR, NIAID
	M. Vaccarezza	Visiting Fellow	LIR, NIAID
	A.M. Masci	Guest Researcher	LIR, NIAID
	U. Siebenlist	Senior Investigator	LIR, NIAID

Cooperating Units:

(D. Kotler); Ctr. Blood Res and Harvard Med. Sch., Boston (T. Springer, A. deFougerolles); Duke Univ. Med. Ctr. Durham NC (B. Haynes); NMRI, Bethesda, MD (S. Kessler); CABM, UMDNJ, RWJMS, Piscataway, NJ (A. Rabson); Univ. Alabama, Birmingham, AL (G. Shaw, M. Saag); Univ. Montreal (R. Sekaly).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00585-03 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenic Mechanisms in Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.I. Cohen	Expert	LIR, NIAID
Others:	H.C. Lane	Senior Investigator	LIR, NIAID
	Y. Tani	Visiting Associate	LIR, NIAID
	H. Tian	Guest Worker	LIR, NIAID

COOPERATING UNITS *(if any)*

CBMB, NICHD (L.Samelson); New York Univ. (S.Zolla-Pazner); UC San Diego (S.Hedrick); UCSan Diego (J.Newport)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.3	3.3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to dissect the molecular mechanisms whereby HIV-1 functionally alters or kills CD4+ T lymphocytes, transfected Jurkat cell lines expressing different HIV-1 proteins were established. Cells constitutively expressing functional gp120 and gp41 showed no direct alterations in their cell growth and did not spontaneously fuse. In contrast, HIV-infected cells or HIV-envelope transfected cells could be induced to form syncytium and die upon co-culture with naive CD4+ cells. Such infected or transfected cells undergoing cell fusion and cell death displayed dramatic alterations in their intracellular signalling pathways as evidenced by changes in tyrosine phosphorylation of intracellular substrates. Blockade of these phosphorylations with an inhibitor of protein tyrosine phosphorylation, herbimycin A, dramatically reduced the cytopathicity associated with HIV-1. While proteins of about 95 and 30 kilodaltons were most dramatically tyrosine phosphorylated during HIV-1 cell fusion, the phosphorylation of the smaller substrate correlated most closely with syncytium formation and cell death. This protein was identified as the cyclin dependent kinase cdc2, a central regulator of cell cycle which is inhibited by tyrosine phosphorylation.

The heavy and light chain antibody genes derived from two human anti-HIV envelope gp41 monoclonal cell lines were cloned, sequenced, and functionally expressed in recipient B cell lines. The genes from one of the two monoclonal lines conferred anti-gp41 specificity to transfected cell lines. The heavy and light chain variable region genes from this antibody (98-6) were genetically linked to the T cell receptor (TCR) constant α and β regions, respectively to generate chimeric antibody/TCR genes. When these chimeric TCR genes were expressed in T lymphocytes, they conferred TCR-dependent activation function to the cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00587-03 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gene Expression of the Human Immunodeficiency Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.I.Dayton	Sr. Staff Fellow	LIR, NIAID
Others:	E.T. Dayton	Staff Fellow	LIR, NIAID
	D.M.Powell	Graduate Student	LIR, NIAID
	J. Lim	Summer Student	LIR, NIAID
	S. Lee	Clinical Associate	LIR, NIAID
	S. Bende	IRTA Fellow	LIR, NIAID
	L. Butini	Visiting Scientist	LIR, NIAID
	G. Pantaleo	Visiting Scientist	LIR, NIAID

COOPERATING UNITS *(if any)*

LMB, NCI (J.V. Maizel); LMB, NCI (B. Shapiro); Univ. Colorado, Boulder, (D. Konings) LTCB, NCI (R. Gallo); LTCB, NCI (M. Klotman); James N. Gamble Inst., Cincinnati, OH (B. Baroudy); James N. Gamble Inst, Cincinnati, OH (G. Kotwal).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have detected in crude extracts a nuclear activity which promotes the binding of Rev to the Rev-response element (RRE). This activity should be crucial for the *in vivo* function of *Rev*. This activity either cooperatively binds to the RRE with *Rev* or catalyzes the binding of *Rev* to the RRE. Almost certainly the activity is protein. Chromatographic analysis of the extracts has resolved at least three and possibly as many as five separate activities. Currently we are purifying the separate activities and cloning them. In parallel with these studies, we have detected the existence of cell-type specific cellular factors by studying the replication kinetics in different cell types of human immunodeficiency virus (HIV) proviruses with RRE mutations. In different cell types, HIV proviruses with different RRE mutations replicate with drastically different kinetics. These data are the first reported indication that the RRE may contribute to cell-type specific viral tropism. In related work we have studied the interaction of the *Rev* protein with the RRE. We have found that RRE elements determine the binding of *Rev* molecules subsequent to the first binding event. This precludes models for *Rev*/RRE binding which hold that all of the specificity is in the first binding event and that subsequent multimerization of *Rev* along an RNA molecule involves only non-specific recognition of RNA by *Rev* protein. This work has also identified RRE molecules that tightly bind *Rev* but that do not permit more than two *Rev* molecules to bind. Functional analysis of theses mutations will assess the requirement of multimerization for function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00390-09

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Anti-retroviral Therapy of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Polis	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Laboratory Chief	LIR, NIAID
	H.C. Lane	Section Head	LIR, NIAID
	R.T. Davey, Jr.	Senior Investigator	LIR, NIAID
	R.E. Walker	Senior Investigator	LIR, NIAID
	J. Falloon	Senior Investigator	LIR, NIAID
	E.A. Seidel	Medical Officer	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH (H. Masur, J.A. Kovacs); NEI, NIH (R. Nussenblatt)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Betheseda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	1.7	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed toward studying the preventive and therapeutic aspects of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). A randomized trial comparing therapy with zidovudine (ZDV) versus interferon-alpha (IFN-α) versus the combination in 180 patients with early HIV-1 infection completed accrual and is currently active. A dose-tolerance study of the combination of IFN-α and dideoxyinosine completed accrual of 53 patients and is now gathering long-term follow-up data. A randomized, double-blinded, placebo-controlled Phase II study of L-697,661 versus ZDV completed accrual of 84 patients using surrogate markers as efficacy parameters and is now undergoing final analysis. A Phase I study of N-acetyl-cysteine was begun and has enrolled 23 patients to determine the pharmacokinetics, bioavailability, maximally tolerated dose, and preliminary efficacy of this agent in the treatment of HIV-infected persons with < 500 CD4 cells/mm^3. A phase I study of CD4-Pseudomonas Exotoxin (sCD4-PE40) in HIV-infected patients was initiated and enrolled 24 patients to determine the pharmacokinetics, maximally tolerated dose, and preliminary efficacy of this agent. Follow-up analysis of a randomized, controlled, masked trial of foscarnet in the treatment of cytomegalovirus retinitis in AIDS patients demonstrated an apparent survival benefit of this therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00634-01 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigation of the SCID-hu Mouse Model of HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.T. Davey Jr.	Senior Investigator	LIR,NIAID
Others:			
	A.S. Fauci	Chief	LIR, NIAID
	K. Ruxrungtham	Visiting Scientist	LIR, NIAID
	H.C. Lane	Section Chief	LIR, NIAID
	D. Cohen	Expert	LIR, NIAID

COOPERATING UNITS *(if any)*

Georgetown University (N. Salzman, M. Psallidopoulos).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.45	1.45	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three different experimental murine animal models of human immunodeficiency virus type 1 (HIV-1) infection were evaluated in SCID mice for their technical feasibility, efficiency and distribution of successful lymphocyte transfer/engraftment, and susceptibility to detectable HIV-1 infection after reconstitution. Sources of reconstituted human cells that were studied included both fresh and frozen (cadaveric) bone marrow, fetal tissue, and peripheral blood. Based upon this analysis it was concluded that one model particularly well-suited to adaptation in the testing of putative antiretroviral agents was the SCID-hu-PBL model involving reconstitution of 8-12 week old SCID mice with fresh peripheral blood mononuclear cells obtained by lymphopheresis from normal donors. Reconstitution of SCID mice by intraperitoneal injection of harvested mononuclear cells led to successful and sustained transfer of lymphocytes that were optimally infectable 2-3 weeks after reconstitution. Human Ig antibody production was demonstrated in reconsituted mice as was trafficking of a small percentage of human lymphocytes to the murine spleen. In a series of experiments the optimal titer of infectious HIV-1 IIIB was determined using viral detection by both lymphocyte co-cultivation and polymerase chain reaction methods. Oral and intraperitoneally-administered zidovudine was found to demonstrate a dose-dependent protective effect in this model. Currently, this model is being adapted for the testing of other putative antiretroviral agents as well as for an assessment of the relative protection afforded by reconstitution with mononuclear cells derived from healthy gp-160-immunized donors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00635-01 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Trials for the Prevention and Treatment of HIV-Associated Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Falloon, M.D.	Senior Investigator,	LIR, NIAID
Others:	R.T. Davey, Jr.	Senior Investigator	LIR, NIAID
	M. Polis	Senior Investigator	LIR, NIAID
	R. Walker	Medical Officer	LIR, NIAID
	J. Metcalf	Biologist	LIR, NIAID
	L. Wood	Medical Officer	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH (H. Masur, J. Kovacs, D. Paar, V. Gill, F. Witebsky, K. Ownby, B. Baird, D. O'Neill, G. Kelly, M. Larson, P. Smith, M. Amantea); Georgetown University Med. Ctr. (J. Lavelle); Univ. of Philadelphia (R.R. MacGregor).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
	1.95	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed toward studying the prevention and treatment of opportunistic infections associated with AIDS. Phase I/II trials of BW566C80 as salvage therapy for cerebral toxoplasmosis demonstrated both long-term safety and efficacy. BW566C80 in combination with pyrimethamine has also proven safe and effective. Azithromycin as salvage therapy for toxoplasmosis resulted in early treatment failures. In data from a trial of foscarnet for the treatment of cytomegalovirus retinitis, higher titers of neutralizing antibody were found to correlate with a longer recurrence-free time on therapy. Thus, a Phase I/II study of the pharmacokinetics of CMVIG-IV for the prevention of CMV disease in patients with AIDS was begun. Data from this study will be used in a planned Phase II efficacy study of this product. Analysis of a multi-center Phase III trial of BW566C80 vs. trimethoprim-sulfamethoxazole for the treatment of pneumocystis pneumonia demonstrated equivalent overall therapeutic efficacy but therapeutic superiority of TMP-SMX with superior safety and tolerability of BW566C80. A study of the pharmacokinetics of BW566C80 confirmed the limited oral absorption of this agent with a plateau of absorption, large inter-patient variability, and drug levels that may be suboptimal for the treatment or prevention of pneumocystis pneumonia in some patients. A pilot study of weekly dapsone and dapsone plus pyrimethamine for the prevention of pneumocystis pneumonia demonstrated the safety of these regimens but also a significant occurrence of break-through episodes of pneumonia. A seven-arm Phase I/II trial of sparfloxacin, azithromycin alone or in combination plus a salvage regimen using these agents with ethambutol and clofazimine for the treatment of Mycobacterium avium complex infection is now ready to enroll. A controlled trial of BW566C80 for the treatment of cryptosporidiosis and microsporidiosis has been substantially completed but has yet to be analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00636-01 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Approaches to the Therapy of HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Walker	Medical Officer	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	H.C. Lane	Section Chief	LIR, NIAID
	R. T. Davey, Jr.	Medical Officer	LIR, NIAID
	J. Falloon	Medical Officer	LIR, NIAID
	M. Polis	Medical Officer	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH (H. Masur, J. Kovacs), DAIDS, MTB (S. Schnittman) Medimmume (S. Krenig) Scripps Institute (D. Burton)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed toward studying the potential therapeutic aspects of immunologic approaches to human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome. Adoptive immunotherapy utilizing peripheral blood lymphocytes and bone marrow from gp160-primed syngeneic donors in association with combination antiretroviral agents was continued. One of three recipients demonstrated persistent elevations in CD4 counts after adoptive transfer. A phase I clinical trial administering a nef-specific CTL clone to its donor with IL-2 infusions was initiated. The CTL infusions were well tolerated, and modest antiretroviral activity was observed. Active immunotherapy protocols utilizing gp160 and p24 immunogens in seropositive patients were continued. Both recombinant proteins were well tolerated and produced lymphoproliferative responses. A study generating random recombinatorial libraries of human immunoglobulin genes from HIV infected individuals was initiated in an effort to produce human monoclonal antibodies for use in passive immunotherapy. Phase I studies of the combinations of zidovudine (ZDV) and interleukin-2 (IL-2) and interferon-α and IL-2 were continued. The maximum tolerated dose of IL-2 when administered with ZDV has been defined, and the study has been modified to evaluate the immunologic and antiviral effects of the repeated administration of these agents. A protocol evaluating the safety and pharmacokinetics of N-acetylcysteine is nearing completion. No new toxicities have been observed, and oral bioavailability appears so low that oral dosing may be impractical. No antiretroviral effects have been seen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00361-10

PERIOD COVERED

October 1, 1990 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

International Studies on the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	C. Brown	Expert	LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins University (E. Hook, A. Rompalo, J. Zenilman, N. Halsey, G. Kelen).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The acquired immunodeficiency syndrome (AIDS) is a global pandemic with over 11 million HIV-infected individuals worldwide. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In Port-au-Prince, Haiti, we demonstrated that nearly 10% of pregnant women are currently HIV-infected and have a 2% annual seroincidence. In a survey in Kinshasa, Zaire, we demonstrated a 40% infection rate in female prostitutes with an estimated 10% annual seroincidence. Among patients attending STD clinics in Baltimore, a retrospective 10-year survey demonstrated a marked increase in HIV infection to 5.5% with a current annual seroincidence of 2.0%. Common to all three cohorts was the strong association of HIV infection among heterosexuals with past or active genital ulcerative and non-ulcerative STDs, smoking, lack of circumcision, and inconsistent use of condoms. We have documented a 28% perinatal transmission rate with transmission associated with depressed maternal CD4$^+$ lymphocyte counts, anemia, chorioamnionitis and funisitis. There was no association between the presence of neutralizing antibodies to the V3 loop of gp120 and perinatal transmission. Following acid association of immune complexes, we were able to demonstrate extremely high levels of p24 antigenemia during the first three months of life in perinatally infected children. When used in combination with either PCR or an IgA assay for HIV-specific antibodies, the diagnosis of perinatal HIV infection could be established within the first three months of life with a sensitivity and specificity of 99%, respectively. The identification of CD4$^+$ and CD8$^+$ CTL clones in volunteers receiving recombinant gp160 vaccines led to the recognition that upon antigen stimulation, these clones were capable of producing high levels of TNF-α which resulted in up-regulation of HIV in latently infected cell lines. Additional studies are planned to further elucidate the elaboration and importance of these CTL clones in the immune response to HIV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00210-12 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulation of Human Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.H. Kehrl	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	Y. Deguchi	Visiting Associate	LIR, NIAID
	J.X. Hong	Visiting Associate	LIR, NIAID
	E. Kozlow	Clinical Associate	LIR, NIAID
	A. Riva	Guest Researcher	LIR, NIAID
	C. Thevenin	Visiting Associate	LIR, NIAID

COOPERATING UNITS *(if any)*

BRB, NIAID (J. Coligan); NIAID, NIH (A. Kirschenbaum); Mount Sinai Medical Center, NY (V. Najfeld, L. Mayer); Gerogetown Medical Center (P. Katz); Yale New Haven Hospital, New Haven, CT (C. Fox).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of the mechanism which regulate the activation, proliferation and differentiation of human B lymphocytes on both a cellular and a molecular level. We have isolated the B cell specific CD22 gene, and characterized its structure, transcriptional start sites, and begun to study the regulation of its expression. We have fully sequenced two novel cDNA clones, BL11 and BL34, which were found to be induced in high levels in activated B cells. Studies are in progress to characterize the genomic structures of these two genes, to generate antibodies against the BL11 and BL34 proteins, and to express these proteins in bacteria and mammalian cells. The proximal portion of the CD20 promoter has been studied and found to contain a diverged Oct-2 binding site which is important in the B cell specific expression of CD20 in mature B cells and the induction of CD20 expression in a pre-B cell line. Mobility shift assays have identified other potentially interesting DNA binding proteins in the more distal CD20 promoter. In vivo foot printing has been established in the laboratory and is being used to characterize the CD20 promoter and will be used to help the analysis of the CD19 and CD22 promoters as well. Analysis of the CD19 promoter is in progress and several B cell lines which are CD19 negative have been established. A cDNA predicted to encode for a novel protein kinase, GCK, which is differentially expressed at the mRNA level, expressed in germinal center but not mantle zone B cells, has been isolated. The human homeobox gene HB24 has been used to generate transgenic mice using the T cell receptor promoter/enhancer. The HB24 transgenic mice have a major defect in $CD4^+$ T cell development and the thymuses of these mice fail to undergo normal thymic involution. Both HB24 and HB9, another human homeobox gene, were found to be expressed at high levels in acute leukemic, but not in chronic leukemic cells. Injection of nude mice with Jurkat cells transfected with HB24 induces tumors while control cells do not. Genomic clones for HB9 and HB24 have been isolated and have been partially characterized. The HB9 and HB24 genes have been co-localized to the long area of chromosome 1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00431-08 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Molecular Biologic Approach to Immune Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U. Siebenlist	Expert	LIR, NIAID
Others:	V. Bours	Visiting Fellow	LIR, NIAID
	K. Brown	Visiting Associate	LIR, NIAID
	G. Franzoso	Visiting Fellow	LIR, NIAID
	T. Kanno	Visiting Fellow	LIR, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS *(if any)*

LP, NCI (K. Kelly); Squibb Inst. Molecular Biol. (R. Bravo).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory is interested in immune activation and, in particular, the molecular mechanisms underlying the activation of T cells, a key step during an immune response. Our initial approach to gain fundamental insights into the early phase of activation of human primary T lymphocytes involved the isolation by subtractive technologies of novel mitogen- and antigen-inducible genes. Characterization of several of the more than 60 genes isolated in this way has led to the discovery of several cytokines, transmembrane proteins, a phosphatase, a ras-related protein and six different transcription factors. Two of these originally cloned factors are part of the NF-κB transcription factor complex called p50 and p50B. NF-κB is an important component of the immune response; it is essential to the regulated expression of many immunomodulatory proteins, acute phase proteins and viruses, including the human immunodeficiency virus (HIV). Upon antigen- or cytokine- or stress-mediated signalling NF-κB is released from a cytoplasmic inhibitor called IκB to translocate to the nucleus. We have determined that NF-κB activity is created from different homo-and heterodimeric complexes which all differ in their functions. The homodimeric p50 complex actually acts as a repressor in the nucleus opposing the transactivating heterodimeric complexes. Both types of complexes are independently regulated by members of the IκB family: IκB inhibits transactivating complexes and the oncoprotein Bcl-3 inhibits the repressing complexes; these inhibitory molecules therefore have opposite effects on gene expression. The complexity of the NF-κB transcription factor suggests an equally complex regulatory role during immune activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 000213-12 LIR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Immunopathogenic, Therapeutic Studies of Vasculitis and Other Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G.S. Hoffman	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	G.S. Kerr	Visiting Associate	LIR, NIAID
	M.C. Cid	Guest Researcher	LIR, NIAID

COOPERATING UNITS (if any)

LCI, NIAID (M. Rottem); NCI, NIH (W.D. Travis); LDBA, NIH (H. Kleinman, D. Grant); NIAID, NIH (C. Hallahan); DMID, NIH (M.F. Cotch, R.A. Kaslow); DAIT, NIH (H.B. Dickler); DIR, NIDCD (R.S. Lebovics).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Vasculitis and Related Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have established a computerized data bank to store, retrieve and analyze an extensive collection (26 years/ >200 patients) of epidemiological, clinical and laboratory information about the systemic vasculitides. We have continued to study the efficacy of daily low dose cyclophosphamide (CP) and prednisone as remission inducing and potentially curative therapy in systemic vasculitis. The long-term toxicity of daily CP therapy in some patients has led to studies of alternative treatment including (1) intermittent intravenous high dose CP, and (2) weekly low dose methotrexate (3) daily trimethoprim/sulfamethoxazole in limited Wegener granulomatosis (WG) and high dose intermittent intravenous immunoglobulin. We have evaluated various surgical interventions for the treatment of upper airway disease in WG. We have studied the sensitivity, specificity and prognostic value of the antineutrophil cytoplasmic antibody to determine its utility as a guide to therapy in WG. Because the vasculitides are rare disorders, clinical research has been limited by the inability of any single institution to recruit large numbers of patients. To address this issue we have organized an 8 site Multi-Center Collaborative Network that will address problems relating to epidemiology and treatment trials. The pathogenesis of the vasculitides has been investigated *in vitro* with the aid of endothelial cell systems to (1) characterize leukocyte binding to normal control and autologous endothelial cells, (2) determine whether sex hormones influence leukocyte-endothelial cell adhesion, a question that is particularly relevant to Takayasu arteritis, and (3) assess the role of haptoglobin in stimulating angiogenesis. An epidemiologic analysis of patients with WG has continued to evaluate environmental factors, such as inhalant exposure and patient clusters.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-10

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenesis of *Chlamydia trachomatis* Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T.C. Quinn Senior Investigator LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins University, (E. Hook, L. Bobo, R. Viscidi).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the US with an estimated 5 million cases annually. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop improved diagnostic assays and to examine the pathogenesis of chlamydial infection in experimental animal models. Studies of genital chlamydial infection in patients attending STD clinics have demonstrated a prevalence of 15% in men and women with and without symptoms. Prevalence varied little by either sex, symptoms, number of sex partners or the patient's relationship to his sex partners, although co-infection with *N. gonorrhoeae* and *C. trachomatis* was quite frequent in this population. Utilization of PCR nearly doubled the sensitivity of culture for the detection of *C. trachomatis* among sexual partnerships. Infection was identified by PCR in 75% of male partners to infected females compared to only 39% by culture. Sequence analysis of amplified chlamydia DNA from partners identified the same serovar and further supported the accuracy of this assay. Utilizing urine specimens from 1,000 asymptomatic men, we identified chlamydia infeciton by PCR in 11%. PCR was also utilized for the detection of chlamydial conjunctival infections in over 200 children with trachoma and demonstrated early relapse in over half of the children following mass therapy within four weeks post-therapy. In an experimental animal model where viability of *C. trachomatis* can be monitored, persistence of chlamydial DNA and RNA was demonstrated in monkeys who had become culture negative. This persistence of chlamydial DNA may possibly contribute to the pathogenesis of chlamydia and appears to be related to the production of interferon γ by the host and elaboration of heat shock protein (HSP-60) by the organism. Clinical studies utilizing primer sets from the 16s rRNA of *C. pneumoniae* have demonstrated a marked increase in sensitivity and specificity of PCR for detection of *C. pneumoniae* in clinical pneumonia. Utilizing this assay, *C. pneumoniae* was identified in approximately 4% of nearly 500 patients with respiratory disease and in none of asymptomatic individuals.

LABORATORY OF MOLECULAR MICROBIOLOGY
1991 Annual Report

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LABORATORY OF MOLECULAR MICROBIOLOGY

SUMMARY

The Laboratory of Molecular Microbiology (LMM) applies molecular and biological techniques to study the structure, function, and regulation of prokaryotic and eukaryotic genes. Programmatically, a wide range (murine retroviruses, the human immunodeficiency virus (HIV), yeast, and mycoplasmas) of microorganisms and their host cells are investigated. Members of the LMM are physically located in Building 4 on the Bethesda campus and in Building 550 at the Frederick Cancer Research Facility. Although the research activities conducted by the LMM staff are quite diverse, a common theme involves the characterization of genetic elements (both chromosomal and episomal) affecting the interaction of microorganisms and the cells they infect.

STUDIES OF THE HUMAN IMMUNODEFICIENCY VIRUS

Molecular biological and virological techniques have been employed to dissect structure/function relationships of structural and regulatory genes of the human immunodeficiency virus (HIV) during acute and chronic virus infections. In contrast to most other groups investigating HIV, LMM scientists have tended to focus on the effects of mutated viral genes in the context of the complete viral genome; complementary *in vitro* analyses of isolated HIV genes have also been carried out.

Structural and functional analysis of the HIV-1 Vpu gene product. The HIV-encoded Vpu protein is an integral membrane phosphoprotein which is present in high concentrations in virus-producing cells but appears to be excluded from virus particles. Vpu forms homopolymeric complexes *in vivo* and *in vitro*; protease protection experiments indicate that Vpu possesses the topology of a Class I integral membrane protein but lacks the signal peptide processing signal. An analysis of potential interactions of Vpu with other viral or cellular proteins demonstrated that it affected the processing of the HIV envelope glycoprotein precursor gp160 and the intracellular stability of CD4. In the absence of Vpu, gp160 and CD4 form stable intracellular complexes which cannot be transported intracellularly. In the presence of Vpu, gp160/CD4 complexes are destabilized and normal synthesis of gp120/gp41 ensues. A series of pulse/chase experiments, designed to investigate the mechanism of the Vpu-dependent destabilization of gp160/CD4 complexes revealed that Vpu induces the rapid degradation of CD4 reducing its half-life from normally 6 hours to 12 minutes. Degradation was shown to depend on the retention of CD4 in the endoplasmic reticulum of the cells which is accomplished by the formation of gp160/CD4 complexes. However, gp160 is not directly involved in the degradation of CD4 and its function as a CD4 trap can be substituted by Brefeldin A, a drug that blocks membrane traffick in the endoplasmic reticulum.

(Willey, Maldarelli, Strebel)

In vitro degradation of CD4: Identification of an active domain in Vpu and a Vpu-responsive element in CD4. An *in vitro* test system was developed to study the Vpu-induced degradation of CD4. Vpu and CD4 were expressed in rabbit reticulocyte lysates in the presence of microsomal membranes to effect membrane association of the two integral membrane proteins and to permit CD4 glycosylation. Co-translation of CD4 and Vpu resulted in reduced levels of CD4 compared to reactions lacking Vpu. The degree of reduction was dependent both on the quantities of Vpu synthesized as well as the incubation time of the experiment. Pre-translation of CD4 prior to synthesis of Vpu reduced the levels of CD4 and supported a model of Vpu-induced CD4 degradation *in vitro*. An analysis of Vpu mutants indicated that deletion of 6 amino acids from a conserved cytoplasmic domain of Vpu eliminates biological activity. Other studies using CD4 mutants demonstrated that Vpu targets the cytoplasmic tail of CD4. Experiments are in progress to investigate whether transfer of CD4 cytoplasmic sequences to heterologous membrane proteins confers sensitivity to Vpu.

(Chen, Strebel)

Structure and Function of the HIV-1 envelope glycoproteins. The HIV-1 envelope glycoproteins, gp120 and gp41, mediate viral attachment and penetration of $CD4^+$ cells. The removal of a single asparagine-linked carbohydrate from gp120 in a region which is highly conserved among different HIV-1 isolates, severely impairs virus replication. However, spontaneous revertant viruses arose during passage of the HIV-1 mutant in tissue culture. Analyses of the revertant viruses indicated that single amino acid changes in other regions of gp120 could compensate for the original defect and restore infectivity to near wild type levels. Analyses of both the mutant and revertant virions demonstrated that the glycosylation mutation reduced the amount of virion-associated gp120 by almost 90% when compared to the parental wild type virus. The amount of gp120 found associated with the revertant virions was 50% that of wild type even though the revertant amino acid changes did not generate asparagine-linked glycosylation sites. Work is currently in progress to determine if the revertant changes affect the neutralization properties of HIV-1 since one of the identified reversions occurred in the principal neutralizing domain (V3 loop) of gp120.

(Willey and Martin)

Identification of a packaging signal in the U5 region of HIV Long Terminal Repeat (LTR). Deletion mutations of the 5', middle, and 3' thirds of the HIV LTR U5 region were constructed in an infectious molecular clone of HIV. Virus stocks were generated following the transfection of the mutated DNAs into Hela cells and their replicative capacities were monitored in susceptible $CD4^+$ T cells. The deletion of the middle third of U5 was dispensable for viral replication, whereas the deletion of the 5' and 3' thirds markedly reduced infectivity. Analyses of the protein and RNA content of the progeny virions produced by these mutant proviruses in Hela cells revealed that the deletion of the 5' third of U5 caused a 10-20-fold reduction in the RNA content of progeny virions despite the synthesis of normal amounts of *gag* and *env* proteins. These data suggest that the 5' end of U5 sequences contains a packaging signal for incorporating genomic RNA into progeny virions.

(Vicenzi and Martin)

Replication of Tat-minus HIV is dependent on LTRs containing a heterologous enhancer element. The biological activity of reconstructed HIV LTRs containing heterologous enhancer elements was examined in the context of both CAT and proviral DNA constructs. LTRs containing CMV promoter/enhancer elements in place of the NFκB and/or Sp1 sites directed the expression of high basal levels of CAT activity in HeLa and human-B lymphoid cells. The same reconstructed LTRs, when present in HIV proviral construct, resulted in the production of progeny virions that were infectious in human PBLs. This high basal level of LTR-directed expression suggested that Tat-minus mutants of such viruses might replicate in CD4 positive T-cells. Indeed, the Tat-minus, NFκB/Sp1 minus, CMV-IE enhancer positive HIV construct produced detectable progeny virus following transfection of HeLa cells. Infectious virus was also produced after infection of human T and B cell lines using the supernatants from transfected HeLa cells. However, the kinetics of infection were markedly delayed (three weeks) compared to wild type virus (one week). Tat-minus HIV with a wild type LTR failed to replicate over a 6 month period. The properties of Tat-minus viruses are being evaluated in different culture systems.

(Chang and Martin)

Efficient expression of the HIV genome in mouse cells. The 5′ LTR was deleted from infectious molecular proviral DNA clones for two HIV-1 isolates and replaced with transcriptional regulatory elements from either simian cytomegalovirus (CMV) or Moloney murine leukemia virus (MuLV). The 3′ LTR was left intact. Constructs containing CMV sequences gave the highest level of expression in mouse cells in culture and, as predicted, were not infectious. Constructs containing MuLV sequences were unstable in mouse cells. Rearrangements occurred in the 5′ LTR that deleted the heterologous MuLV sequences, and substituted altered versions of the 3′LTR, giving rise to infectious virus. Since one aim of this study is to establish a better animal model for HIV induced pathogenesis, the CMV recombinant proviral genome has been microinjected into mouse embryos to generate transgenic mice. Unfortunately, the litter size of microinjected embryos has been consistently small, suggesting that a high level of CMV directed HIV expression may be occurring during fetal development resulting in intrauterine death.

(Mounts and Martin)

Cell-to-cell spread of HIV. A sensitive system to monitor the cell-to-cell transmission of HIV-1 has been developed which utilizes chronically infected H9 donor cells. Studies with this system have shown that virus induced cell-cell fusion occurs within 15 to 30 minutes of co-culture as monitored by electron microscopy, sCD4 competition and dye-diffusion analysis. This system has also been used to evaluate HIV-1 infection of activated and resting human PBLs. Activated unfractionated PBLs synthesize viral DNA 4 to 6 hrs. following incubation with infected H9 cells and produce progeny virions by 22 hrs. post infection. Viral DNA is also produced in resting unstimulated PBLs (to approximately 40% the level observed in activated cells) but no detectable progeny virions are produced. Linear as well as circular forms of viral DNA are synthesized in resting PBLs indicating that the nuclear transport of reverse transcripts is not inhibited. Studies are underway to ascertain whether the integration step of the virus life cycle is blocked in unstimulated PBLs.

(Sato and Martin)

The contribution of TATA elements in LTR-directed gene expression. *In vitro* mutagenesis techniques have been used to alter a variety of *cis*-acting elements located in the HIV-1 long terminal repeat (LTR). When examined in the context of an infectious molecular proviral DNA clone, elimination of the NFkB sites had little effect on virus infectivity for human PBLs as well as several T cell lines. In contrast, deletion of the three Sp-1 sites (Sp-1_{DEL}) eliminated HIV replication in some T cell lines and markedly retarded virus growth in others, including human PBLs. Two types of second-site revertant changes involving the TATA motifs appeared in an ordered fashion following long-term passage of the Sp-1_{DEL} mutant virus. Substitution of the TATA box changes into the Sp-1_{DEL} virus restored infectivity. Transcriptional studies of the mutant and revertant LTRs indicate that basal levels of transcription have been augmented (2 to 3 fold) explaining the augmented replicative properties conferred by the altered TATA motifs.

(Ross and Martin)

Quantitation of HIV-1 infection kinetics. A series of equations have been derived describing the kinetics of HIV-1 accumulation as a result of cell-free virus infections or transmission of virus during multiple rounds of infection via cell-to-cell spread. An infection rate constant ($k = \ln n/t_i$) was determined from a series of HIV infections of CEM, H9, and PBLs carried out at different multiplicities of infection. In this derivation, n is the number of infectious units produced by one cell (which ranged from 60 to approximately 220, depending on the cell type) and t_i is the time it takes to complete a single cycle of replication (which was usually 3 days in all cells examined). Of particular note were findings which showed that the "infectivity" of virus producing cells (cell-to-cell spread) was 100 to 1000 times greater than the infectivity of any of the cell-free virus stocks tested.

(Willey, Dimitrov, Martin)

Isolation of an infectious molecular clone of a macrophage-tropic HIV-1 isolate. A full-length infectious molecular clone of the macrophage tropic virus strain, HIV_{AD-87} was obtained from infected human PBLs. The virus produced following transient transfections of HeLa cells replicated in monocyte derived macrophages (MDMs) and human PBLs but not in continuous T-cell lines. Procedures were developed for the differentiation and propagation of MDMs in the absence of added cytokines. When used in infectivity assays, virus yields, ranging from 33 to 75% of those observed with MDM cultures supplemented with M-CSF, were obtained. Mutations are currently being introduced into the IN gene of the HIV_{AD-87} cloned provirus to ascertain whether integration is required for replication in MDMs. Future projects will involve the mutagenesis of the *vif, vpr, and nef* genes to evaluate their function in MDMs.

(Theodore, Englund, Martin)

Identification of HIV-1 isolates capable of infecting chimpanzee PBLs. At the present time, the chimpanzee is the only animal other than man that is susceptible to infection by HIV-1. Thus far, the inoculated animals fail to develop disease. An overwhelming majority of virus samples inoculated into chimpanzees have been derivatives of the $HIV\text{-}1_{IIIB}$ isolate which is incapable of entering and infecting monocyte derived macrophages. We have therefore begun isolating samples of HIV-1 from seropositive individuals with a diagnosis of AIDS assuming that they may have circulating macrophage tropic strains of HIV-1 in their peripheral blood. Virus has been isolated from 23 of 25 individuals; in three instances, samples were obtained by cocultivation with human PBLs, that can replicate efficiently in chimpanzee PBLs. In one case, cell-free virus could be used to infect the chimp cells immediately following its isolation; in two other instances, the introduction of HIV into chimp cells by cocultivation was required before cell-free virus stocks, capable of infecting chimp cells, were obtained. These primary HIV-1 isolates will be used to infect chimpanzees.

(Hoggan and Martin)

Identification of inhibitory regions (IR) elements within HIV genomic RNA. A sensitive reporter plasmid, which employs the HIV *tat* gene, has been developed to identify sequences present in the viral genome that inhibit expression of HIV mRNAs. Two independent elements were identified: one in the *gag* and one in the *pol* gene. The inhibitory effect of these elements was verified in a heterologous plasmid containing the chloramphenicol acetyl transferase (CAT) gene, and was found to be orientation-dependent. IR-containing mRNAs were detected as spliced and unspliced transcripts in the nucleus, but not in the cytoplasm of transfected cells, indicating that the inhibition of expression is a post-transcriptional event. To analyze whether Rev could "rescue" expression of IR-containing mRNAs, recombinant CAT plasmids were constructed which contained the IR sequence and the Rev-responsive element (RRE), a sequence within the HIV *env* gene to which Rev binds. Rev permitted the expression of CAT reporter plasmids containing both IR and RRE sequences; no effect of Rev was observed on the expression of CAT plasmids containing only the IR or the RRE sequences. The inhibitory effect of IR sequences was observed in several primate cell lines but was not demonstrable in mouse cells. Studies are in progress comparing human and mouse cell lines to identify specific cellular factors responsible for the Rev-dependent regulation of HIV gene expression.

(Maldarelli, Martin, Strebel)

Regulation of splicing of HIV mRNA. Alternative splicing of RNA is a mechanism that is used to regulate gene expression during cell growth and differentiation. The role of this mechanism has been examined in regulating the replicative capacity of HIV. Many of the the splice donor and acceptor motifs in the infectious clone of HIV, pNL4-3, have been mutated, yielding viruses that fail to produce the full complement of spliced mRNAs. Analyses of the biological and biochemical properties of these viruses indicate that they exhibit patterns of protein expression and altered growth kinetics despite the apparent redundancy of splicing signals. A semi-quantitative PCR assay has been developed that determines the relative proportions of alternatively spliced HIV mRNA species during infection. This assay is being used in conjunction with mutagenesis studies to examine HIV mRNA expression in different cell types and correlate the expression of alternatively spliced mRNAs with the biological and biochemical properties of HIV generated in these cells.

(Purcell and Martin)

Characterization of the 41.2 transdominant interfering HIV-1 envelope glycoprotein mutant. A mutation (41.2) in the HIV-1 transmembrane (TM) envelope glycoprotein that dominantly interferes with HIV-1-induced membrane fusion and infectivity has recently been described The properties of the HIV-1 envelope glycoprotein that enable this mutant to dominantly interfere are being investigated by constructing double mutants which contain the 41.2 mutation and a variety of mutations elsewhere in gp120 and gp41 (i.e. in the V3 loop of gp120, the gp160 processing site, the transmembrane domain and cytoplasmic tail of gp41). The equivalent of the 41.2 mutation has also been introduced into the TM envelope glycoprotein of HIV-2. Mutations which cannot revert to wild-type via a single nucleotide change are also being introduced into the amino terminus of HIV-1 gp41. The resultant mutant envelope glycoproteins will be analyzed for their ability to dominantly interfere and will be introduced into the full-length HIV-1 proviral clone pNL4-3.

(Freed and Martin)

Analysis of HIV-1 envelope variable region interactions. A number of chimeras have been constructed between the env genes of the pNL4-3 and WMJ-2 clones of HIV-1. A chimera in which the first and second variable regions (V1/V2) of gp120 have been exchanged between pNL4-3 and WMJ-2 is defective for envelope precursor processing and envelope-induced syncytium formation. This result suggests that an intramolecular interaction between V1/V2 and other envelope domains is required for proper envelope glycoprotein processing. The defective chimeric envelope has been introduced into the full-length HIV cloned provirus, pNL4-3, and revertant viruses (which replicate efficiently in CEM cells) have been obtained. The position of changes which compensate for the original processing defect will be determined.

(Freed and Martin)

Analysis of the function of Vif from HIV-1. Vif mutants of the LAI, MAL, and ELI strains of HIV-1 exhibited delayed infection kinetics in several CD4-positive cell lines compared with the corresponding wild type viruses, but were nonetheless able to be transmitted by cell-free infection . The H9 cell line was refractory to infection by Vif mutants using either cell-free virus or chronically infected H9 cells as inocula. Peripheral blood mononuclear cells were also resistant to cell-free infection by Vif mutants but could be infected by co-cultivation. Virion proteins purified from wild type HIV-1 and Vif mutants were indistinguishable. Pulse-chase experiments failed to reveal significant differences in the processing and release of the major HIV-1 structural proteins gp120 envelope and p24 capsid protein. Therefore, no gross defect in the structure and processing of the virion proteins in Vif mutants of HIV-1 can be demonstrated.

(Fan and Peden)

Changes in HIV-1 associated with adaptation to growth in tissue culture cell lines. Virus derived from the infectious molecular clone pELI1 and adapted by passage to grow in the H9 T cell line, exhibited enhanced replicative capacity and broadened host range. Both the gp120 and the gp41 components of the viral envelope gene developed sequence changes detected by PCR and the single strand conformational polymorphism (SSCP) technique. The mutations identified mapped to one of the regions of gp120 thought to be involved with receptor binding and to the amino terminal portion of gp41, adjacent to the fusogenic domain. When introduced into the original pELI1, these two mutations conferred the enhanced replicative capacity. By separately introducing these mutations into the infectious pELI proviral clone and examining the properties of the resulting viruses, it could be shown that the mutation in gp41 was the initial change and that the additional mutation in the CD4-binding region of gp120 occurred through subsequent selection. These results demonstrate that several determinants are likely to be involved in cell tropism and that selection for viruses with increased replicative capacity can occur in the absence of immune pressure.

(Fujita, Silver, Peden)

REV binding is the primary function of the REV responsive element (RRE). HIV REV is an RNA binding post-transcriptional transactivator nuclear protein. The cognate RNA target of REV is contained within a highly structured, 244 nucleotide RNA located in the viral *env* gene. Direct interaction of REV with the RRE RNA is not an absolute requirement for trans-activation. When the REV binding subdomain of RRE was replaced by the MS2 phage operator sequence (RREZ-MS), REV activated mRNAs containing the heterologous sequence is directed to the operator via the RNA binding motif of the MS2 phage coat protein (MS-C) as a fusion protein, REV/MS-C. REV binding is the primary function of RRE, and cellular factors specifically reacting with RRE are not necessary for REV function.

(Venkatesan, Park, Nam)

An intact amino terminus on the Rev protein is required for trans-activation of RRE containing RNAs, but is partly dispensable for an heterologous RNA target. While REV can be tethered to viral RNAs via an RNA binding motif, the structural integrity of the N-terminus of REV encoding the RRE RNA binding, protein oligomerization and nucleolar targeting functions were still required for maximal trans-activation of non-RRE targets. Deletions and amino acid substitutions in the RRE binding domain of REV abolished the transactivation potential of REV/MS-C for both RRE and RREZ-MS. Insertion of the RNA binding domain of REV near the N-terminus of MS2 in a REV/MS-C protein, mutated at the RNA binding motif of REV, partially relieved the defect of this REV/MS-C mutant for RREZ-MS but not for RRE.

(Venkatesan, Gerstberger, Park, Nam)

An RRE binding cellular factor, RRBF, is identical to the TAR binding protein, TRBP and has a broad range of RNA affinities. A commercial (Stratgene Corp.) HeLa cell cDNA expression library in *E. Coli* was screened with radiolabeled RRE RNA. Two positive recombinant phages, RRBFI and RRBFII, encoding major protein species of 45 and 48 kDal and reacting specifically with RRE RNA, were obtained after four successive screenings. The translated sequence of RRBFI was identical to the previously reported TAR binding protein (TRBP). RRBF was purified to near homogeneity and its RNA binding specificities were evaluated. RRBF displayed a broad range of RNA affinities (in decreasing order) for RexRE of HTLV I, HIV-1 RRE, RRE lacking the REV recognition domain, adenovirus VA

RNA, TAR RNA, and MS2 operator RNA. In transient transfection experiments in HeLa cells, RRBF activated the expression of SV40 early promoter linked gene(s) by 25 fold. However, RRBF expression had no effect on the basal or the TAT activated transcription directed by the HIV-1 LTR or on REV dependent GAG expression from an HIV-1 LTR linked GAG-RRE RNA.

(Park, Nam, Venkatesan)

HIV-1 NEF protein modulates the cell surface expression of the CD4 receptor. HIV NEF protein decreases the surface expression of CD4 receptor both in the $CD4^+$ cells and non-lymphoid cells. The Nef-induced CD4 effect was due to accelerated recycling and degradation of the surface CD4. CD4 molecules, which were retained in the ER or lacking the cytoplasmic domain, were not regulated by NEF. CD4/CD8 chimeras, containing the extra cellular domain of CD4 fused to the intracellular domain of CD8, were modulated by NEF. Myristoylation of NEF was required for the effect on CD4. The NEF effect on the CD4 in T cells may have functionally important effect(s) on T cell activation.

(Hiller, Venkatesan)

Characterization of the HIV-1 LTR target for Tat. Transient transfection experiments using a minimal promoter construction that contains only the HIV-1 TATA element and a downstream TAR sequence was not responsive to Tat. However, the addition of tandem copies of enhancer motifs was sufficient to confer Tat responsiveness to this minimal promoter. In studying the relative responsiveness of enhancers to Tat, a hierarchical order of Sp1> Ap1 > Oct > NFκB was determined. These results were explored further *in vitro*. Here, cross-linking and physical association studies showed that Tat, purified from *E. coli*, can specifically interact with purified Sp1, isolated from HeLa cells. This suggests that a functional protein-protein complex between Tat and Sp1 may exist inside the cell.

(Gatignol and Jeang)

La autoantigen binds TAR RNA. By affinity screening, a second TAR RNA-binding protein has been isolated from an expression HeLa cell cDNA library. Direct sequencing of this cDNA revealed that it encodes the human autoantigen La. Using purified human La, it was demonstrated that its binding constant for TAR RNA is comparable to that of Tat for TAR (20 nM). Mixing of La and TAR RNA together produced a complex that was efficiently immunoprecipitated by anti-La sera. A series of mutant La proteins was generated, some of which failed to bind to TAR RNA. When these mutants were tested for binding to hY1 RNA, the authentic cellular RNA substrate for La, a direct correlation was found between mutants that failed to bind hY1 and those that failed to bind La. These results suggest that La binds TAR RNA through the same protein domain as is used to bind hY1 RNA.

(Chang and Jeang)

Ribozymes targeted against the HIV-1 genome. Ribozymes are specific molecular probes that can be directed against RNA sequences. One ribozyme, directed against a U5 sequence, has been inserted into an infectious molecular clone and inhibited HIV replication. Propagation of this enfeebled molecular clone in tissue culture generated "escape mutants" that have been molecularly characterized. Sequencing of relevant regions of these mutants demonstrated changes in both ribozyme and the RNA substrate regions of the virus. Besides the U5 ribozyme, three other ribozymes targetting the HIV-1 *pol*, RRE, and the R region of the LTR have also been characterized. *In vitro* studies indicate that each is able to cleave its cognate target. Each of these four ribozymes has also been transferred into amphotropic retroviral vectors; studies are underway to ascertain if these vectors produce sufficient catalytic RNA to suppress HIV replication.

(Dropulic, Martin, Jeang)

Development of a highly sensitive reverse transcriptase assay. The sensitivity of the reverse transcriptase (RT) assay has been improved about 100-fold by qualitative and quantitative changes in the reaction mixture. An additional 6 fold increase is obtained by a new simple and rapid method of capturing 30 ul of RT DNA sample in specially coated 96 well microtiter plates instead of spotting 5 ul on DEAE paper. The overall sensitivity of the new RT assay is within 5 fold of the p24 antigen capture assay.

(Repaske)

OTHER RETROVIRUSES

Mutational analysis of HTLV-I Tax and the Tax-responsive LTR targets. The structure/function relationship of 47 mutant Tax proteins have been analyzed in detail. Of the 47 mutants examined, 43 contain single amino acid changes. Each Tax mutant has been introduced into an eukaryotic expression vector and evaluated for its capacity to activate expression from either the HTLV-I or the HIV-1 LTR. Four separate Tax domains have been defined from these studies: a zinc-binding domain which maps between amino acids 23 to 49; a nuclear localization domain which maps between amino acids 2 to 58; two activation regions at the N- and C- termini that are necessary for the trans-activation of the HTLV-I; and a region in the middle of Tax that is required for activation of the HIV-1 LTR.

(Semmes, Jeang)

Identification of a candidate molecular clone for the mouse Friend virus resistance locus, *Fv-2*

The Friend SFFV envelope glycoprotein (gp55) stimulates erythroblastosis by binding to erythropoietin (Epo) receptors. Mice homozygous for the recessive *Fv-2* resistance allele are resistant to Friend viral erythroleukemias and Epo induced erythroblastosis. It had been previously shown that *Fv-2* does not encode the Epo receptor suggesting *Fv-2* encodes some other component involved in signal transduction through this receptor. A candidate for *Fv-2* was identified during efforts to map several common integration sites for Friend helper MuLV in erythroleukemias. One of these genes, *Fre-2*, mapped to the region of chromosome 9 carrying *Fv-2*. To establish the proximity of *Fre-2* and *Fv-2*, a second cross was generated which could be typed directly for both *Fre-2* and *Fv-2*, and multiple DNA markers were typed in this region. The results obtained have produced a molecular genetic map around *Fv-2* and indicate that *Fre-2* is at or very near to *Fv-2* since no recombinants between *Fre-2* and *Fv-2* were found,. Experiments are in progress to characterize this locus further.

(Chakraborti, Kozak)

The mouse *Fv-1* locus. The *Fv-1* restriction locus in the mouse is responsible for the *in vivo* and *in vitro* resistance to subtypes of mouse-tropic murine leukemia viruses. *Fv-1*^{n} mice restrict B type viruses, and *Fv-1*^{b} mice restrict N type viruses. N and B type murine leukemia viruses differ in two adjacent amino acids in CA*gag*. Site specific mutagenesis has been used to generate viral genomes which differ in only one of these two sites, and indicate that only a single amino acid substitution is necessary and sufficient to convert an N-tropic virus to a B-tropic virus. Additional substitutions made at this same site have been analyzed and all 4 novel mutations tested thus far produce replication competent viruses, some of which are B-tropic and some of which are N-tropic. One mutant exhibited a unique phenotype not observed in naturally-occurring viruses. This virus replicates well only in the SC-1 mouse cell line and cells derived from some wild mouse species.

(Chakraborti, Kozak)

Genetic mapping and potential receptor function of the mouse homolog of the human gibbon ape leukemia virus receptor. The mouse homolog of the human gene for the gibbon ape leukemia virus (GALV) receptor (*Glvr*-1) was mapped to mouse chromosome 2 using an interspecies genetic cross. Mouse chromosome 2 also encodes a receptor (*Rec-2*) for the mouse-tropic wild mouse virus M813. Southern blot hybridization, utilizing GALV-derived *env* and *pol*-*env* probes, failed to detect any homology between GALV and M813, but did show that all mouse species carry numerous copies of GALV-related sequences. This result suggests that a functional receptor for GALV-related viruses was expressed during Mus evolution. Human cells transfected with the mouse *Glvr-1* gene have also been tested for susceptibility to M813. Results thus far have been negative, perhaps reflecting the low levels of human *Glvr-1* in these cells.

(Kozak, Adamson, Silver)

Genetic linkage studies in the mouse The characterization of the mammalian genome requires the production of a high density linkage map of various model species. Various mouse genes have already been positioned in the linkage map using somatic cell hybrids and interspecies, and intersubspecies genetic crosses. The progeny of these crosses have now been typed for over 350 loci on 20 linkage groups. Some of the genes mapped most recently include those specifically expressed in nervous tissue, heat shock proteins, calcium channel genes, the homologue of the cystic fibrosis transmembrane conductance regulator, and numerous genes encoding transcription factors. The map locations for several of these genes suggest they may be candidates for specific mouse mutations: thus, one locus specifically expressed in melanocytes maps near the silver coat color locus on mouse chromosome 10, and one locus, *Snap*, synaptosomal associated protein, was mapped near several neurological mutations on chromosome 2. Analysis of these mutant mice showed that *Snap* sequences were completely deleted in *coloboma* mutant mice, suggesting a molecular basis for this mutation.

(Kozak, Adamson)

Cellular genes involved in MuLV-induced neoplastic Disease Nonacute MuLVs can transform mouse cells following activation of proto-oncogenes by insertional mutagenesis. Our studies have described four new common viral integration regions disrupted during tumor induction by two different viruses. 1) Three insertion sites for Friend MuLV in erythroleukemias have been identified. One of these sites mapped near, but not at, the proto-oncogene *Kras-2*; one mapped at *Fli-1*, a previously described common integration site; the third mapped at or near *Fv-2* (see Below). 2) Two novel integration sites, *Vin-1* and *Vin-2*, disrupted by a radiation leukemia virus in T-cell leukemias, were mapped to opposite ends of mouse chromosome 6.

(Kozak)

Fv-4 Resistance. T he Fv-4 gene is a major determinant of sensitivity and resistance to murine retroviruses. Certain wild mice inherit the resistance allele at Fv-4 and are nearly completely resistant to retroviral infection. Several years ago it was found that Fv-4 resistance was tightly linked to, or possibly the same as, an inherited retroviral envelope gene. This envelope gene was cloned in the LMM and found to consist of a truncated retrovirus whose expression was controlled by an upstream cellular promotor. Transgenic mice were constructed to investigate the *in vivo* effects of this gene. Two mouse strains were obtained which carry transgenes at single sites in the genome and express the transgene in relevant tissues. These mice are markedly, but not absolutely, resistant to challenge with Friend murine leukemia virus. This system provides reagents which may be valuable for designing gene therapy approaches for inducing resistance to retroviruses. In addition, since the Fv-4 promoter induced high levels of expression of the Fv-4 envelope gene in transgenic mice, derivative constructs may be useful for expressing HIV genes in mice for studies of pathogenesis.

(Dickie, Limjoco, Fujita, Silver)

Potential *Fis-1* oncogene identified. Several years ago a peculiar cluster of integration sites was found for Friend murine leukemia virus in lymphoid and myeloid leukemias induced by that virus (*fis-1*). In other retroviral systems the finding of such "common integration sites" has signaled the presence of nearby oncogenes, but in the Friend system no transcribed gene could be found in this region. The homologous region of DNA in man was shown to be amplified in several solid tumors and rearranged by chromosome translocation in some B cell lymphomas (*bcl-1*). Recently, a candidate oncogene in this region was identified: a member of the cyclin family of genes implicated in regulating the cell cycle. In collaboration with a research group in London, the *cycD1* gene was shown to be within several hundred kilobases of *fis-1* by pulse field gel analysis, and tumors with proviruses at *fis-1* express *cycD1* RNA, whereas corresponding normal tissue and tumors, without proviruses at *fis-1*, do not express *cycD1* RNA. Thus, *cycD1* may be the elusive *fis-1* oncogene. To further analyze this interesting chromosomal region, two large pieces of mouse DNA, cloned in yeast artificial chromosomes (YAC), were recently obtained by screening a mouse YAC library; one of them also contains *cycD1* sequences.

(Silver)

Common integration site for Friend virus in erythroleukemias involves a member of the *ets* gene family. Using inverse PCR methods developed in the LMM, a common integration site for Friend virus was identified in Friend virus-induced erythroleukemias. This region was found to be the same as a common integration site in non-B, non-T-cell lymphomas induced by Cas BrE MuLV, and the *fli-1* integration site identified in Friend virus-induced erythroleukemias. This region is known to contain a member of the *ets* gene family, now implicated in leukemogenesis by several murine retroviruses.

(Silver, Sels)

MYCOPLASMAS

New Mycoplasma Species from HIV-Positive & AIDS Patients. Taxonomic characterization of a new mycoplasma from the urine of both HIV-positive asymptomic and AIDS patients has been carried out in collaborative studies. Two of twelve isolates have been examined in serologic assays using antisera to more than 100 distinct *Mycoplasma* species. The newly identified organism is highly cytopathic for a variety of eukaryotic cells, including human-derived lymphocytes. This cytopathicity appears related to a unique attachment structure and the ability of the organism to induce intracellular infections. The organism has been designated *Mycoplasma penetrans*.

(Tully)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00027-25-LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joseph G. Tully	Head, Mycoplasma Section,	LMM,	NIAID
Others:	David L. Rose	Research Microbiologist,	LMM,	NIAID

COOPERATING UNITS *(if any)*

Collaborating Units: J.B. Baseman, Univ.Texas, San Antonio, TX; J.M. Bove, Univ.Bordeaux,France; S.C. Lo, AFIP, Washington, DC; A. Yechouron, Hospitalier Verdun, Quebec,Canada.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Mycoplasma Section

INSTITUTE AND LOCATION

NIAID, NIH, Frederick, Maryland 21702

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
Total Man-Years 2.0	Prof. 1.0	Other: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of *Mycoplasma genitalium* in human disease has been difficult to define. The organism is frequently mixed with *M. pneumoniae* and these two organisms share considerable immunologic reactivity. Thus, conventional complement-fixation tests or new immunosorbent assays for *M. pneumonia* infections can not differentiate serologic responses to each organism. We now have also identified mixed *M. genitalium/M. pneumoniae* strains in the synovial fluid of a patient with polyarthritis following primary pneumonia infection, suggesting that *M. genitalium* can become disseminated from either urogenital or oropharynx sites. We have currently defined an indirect immuno-fluorescence assay that will accurately distinguish colonies of each organism on agar plates when mixed strains are grown in culture. This test is being applied to specimens being compared in culture versus direct testing with a variety of molecular probes.

Collaborative studies have also established that animal mycoplasmas (*M. arginini*) can induce severe and fatal infections in immunocompromised patients. The inability of antibiotic drugs to control such infections is related to the mycoplasmastatic activity of known antibiotics and the contribution of an intact humoral and cell mediated defense in the host.

Taxonomic characterization of a new mycoplasma from the urine of both HIV-positive and AIDS patients has been carried out in collaborative studies. The high cytopathogenicity for a variety of eukaryotic cells appears related to the unique attachment structure on the organism and the ability of the organism to induce intra-cellular infections. The organism has been designated *Mycoplasma penetrans*.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00190-14-LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Genetics of Eukaryotic Cells and Their Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. A. Martin	Chief	LMM, NIAID
Others:	E. Vicenzi	Visiting Associate	LMM, NIAID
	Peter Dickie	Visiting Associate	LMM, NIAID
	Hironori Sato	Visiting Associate	LMM, NIAID
	Damian Purcell	Guest Researcher	LMM, NIAID
	Elizabeth Ross	Biologist	LMM, NIAID
	Eric Freed	Staff Fellow	LMM, NIAID

COOPERATING UNITS *(if any)*

Dimiter Dimitrov	Visiting Scientist	DCBD, NCI

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	6	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alternative splicing of RNA is a mechanism that is used to regulate gene expression during cell growth and differentiation. The role of this mechanism has been examined in regulating the replicative capacity of HIV. Many of the splice donor and acceptor motifs in the infectious clone of HIV, pNL4-3, have been mutated, yielding viruses that fail to produce the full complement of spliced mRNAs. Analyses of the biological and biochemical properties of these viruses indicate that they exhibit patterns of protein expression and altered growth kinetics despite the apparent redundancy of splicing signals. A semi-quantitative PCR assay has been developed that determines the relative proportions of alternatively spliced HIV mRNA species during infection. This assay is being used in conjunction with mutagenesis studies to examine HIV mRNA expression in different cell types and correlate the expression of alternatively spliced mRNAs with the biological and biochemical properties of HIV generated in these cells.

A sensitive system to monitor the cell-to-cell transmission of HIV-1 has been developed which utilizes chronically infected H9 donor cells. Studies with this system have shown that virus induced cell-cell fusion occurs within 15 to 30 minutes of co-culture as monitored by electron microscopy, sCD4 competition and dye-diffusion analysis. This system has also been used to evaluate HIV-1 infection of activated and resting human PBLs. Activated unfractionated PBLs synthesize viral DNA 4 to 6 hrs. following incubation with infected H9 cells and produce progeny virions by 22 hrs. post infection. Viral DNA is also produced in resting unstimulated PBLs (to approximately 40% the level observed in activated cells) but no detectable progeny virions are produced. Linear as well as circular forms of viral DNA are synthesized in resting PBLs indicating that the nuclear transport of reverse transcripts is not inhibited. Studies are underway to ascertain whether the integration step of the virus life cycle is blocked in unstimulated PBLs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

01 AI 00218-11-LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and Chemical Studies on Retroviral DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roy Repaske	Research Chemist	LMM, NIAID
Others:	Malcolm A. Martin	Chief	LMM, NIAID
	Keith Peden	Expert	LMM, NIAID
	Klaus Strebel	Sr. Staff Fellow	LMM, NIAID
	Elisa Vicenzi	Visiting Associate	LMM, NIAID

COOPERATING UNITS *(if any)*

Janet Hartley	Research Microbiologist	LVD, NIAID
Paul Torrence	Chemist	LMC, NIDDKD

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The presence of HIV-1 is determined by detection of p24 viral antigen in an antigen capture assay or by detection of reverse transcriptase activity. The p24 assay is about 2000 fold more sensitive than previous RT assays. We have modified the RT assay by making qualitative and quantitative changes, and have devised a DNA capture system which permits quantitative capture of RT DNA from 30 µl of the reaction instead of the 5 µl usually captured on DEAE paper. Overall the current RT assay is about 5 fold as sensitive as the p24 assay. This RT assay is a simple assay, is reproducible, and is more reliable and much less expensive than the p24 assay. Since RT activity is directly proportional to time of reaction, to TTP concentration in the range used and to virus concentration, the sensitivity desired can be selected and results from different laboratories can be equated. A method for successfully cloning 12D7 cells in Terasaki microwell plates with an efficiency of approximately 10% was devised. Individual cloned cell lines of 12D7 cells transfected with two different triple mutant HIV-1 constructs have been obtained. Both constructs have a *gag-pol* frame shift and an *env* deletion in addition to one of two RT mutations which render the transcribed RT RNase H negative. Tests are being conducted to determine if, as a result of infection of these transfected cells by competent HIV-1, a transdominant packaging of defective RT occurs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00300-11-LMM

PERIOD COVERED

October 1, 1992 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Aspects of Viral Oncogenesis in Wild Mouse Species and Laboratory Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.A. Kozak	Res. Microbiologist	LMM, NIAID
Others:	A. Chakraborti	Visiting Associate	LMM, NIAID
	J. Silver	Medical Officer	LMM, NIAID

COOPERATING UNITS *(if any)*

R. Friedrich	Inst. for Med. Virol. West Germany
P. Jolicoeur	Cancer Research Inst., Montreal, Canada
M.T. Davisson	The Jackson Laboratory, Bar Harbor, ME

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mouse genome contains various genes which are involved in virus-induced neoplastic disease. These genes include endogenous retroviral sequences as well as mouse cellular genes which facilitate or restrict virus replication. Various cellular genes can also function as viral receptors; we used the human gene for the gibbon ape leukemia virus (GALV) receptor to identify and map homologous sequences to mouse chromosome 2, suggesting a possible identity with *Rec-2*, the chromosome 2 encoded receptor for a wild mouse retrovirus; we also identified multiple copies of GALV-related sequences in the mouse genome suggesting that this gene may have functioned as a receptor during the evolution of Mus.

In other experiments we identified several novel common viral integration regions associated with tumor induction. Two integration regions for BL/LV3 radiation leukemia virus were mapped to opposite ends of mouse chromosome 6. One of the common integration sites for Friend MuLV mapped near the protooncogene locus Kras-2, and and a second Friend site maps at or near the *Fv-2* resistance gene. In order to determine the possible identity of these genes we mapped both in the same genetic cross. These experiments produced a molecular genetic map around the *Fv-2* locus and failed to provide evidence that the two may be distinct loci.

Finally, we have used site specific mutagenesis to alter the site in the CA*gag* region of the AKV MuLV which is thought to interact with the gene product of the *Fv-1* resistance gene. Results show that only a single amino acid substitution is sufficient to alter virus tropism. Other changes were introduced into this site, some of which did not alter virus replication patterns, some of which converted the original N-tropic virus into a characteristic B-tropic virus, and one of which produced a virus with a novel replication pattern on cells derived from various inbred strains and wild mouse species.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00301-11-LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic mapping of mouse chromosomal genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. A. Kozak	Res. Microbiologist	LMM, NIAID
	M. C. Adamson	Chemist	LMM, NIAID
	R. Callahan	Section Chief	LTIB, NCI
	E. Appella	Section Chief	CCB, NCI
	H. Chin	Sr. Staff Fellow	LMB, NINDS

COOPERATING UNITS *(if any)*

D. Farber; C.Y.F. Lau; D. Joseph; G. Krissansen; N. Moschonas; J. Marth: S. Pestka; J. Gusella; L. Yu; I. Kirsch; N. Hecht; K. Kelley; B. Kwon; M. Wilson.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the major goals of the effort to characterize the human genome is the production of high density genetic linkage maps of model species such as the mouse. Our mapping efforts rely on two different approaches. Traditionally we have used Chinese hamster x mouse somatic cell hybrids to assign newly identified genes to specific chromosomes. These hybrids continue to be useful to determine the number of genes identified by hybridization probes with multiple genomic copies, like the heat shock protein genes, or to map genes which govern species specific traits expressed in tissue culture like the accessory factor for interferon gamma receptor. More recently, we have been mapping genes using Southern blot analysis of the progeny of two genetic crosses, an interspecies and an intersubspecies backcross. DNAs from the progeny of these crosses have been typed for a variety of polymorphic reference loci to permit mapping of unknown markers to specific positions on the linkage map. These studies have resulted in the chromosomal mapping of a large number of genes including the various genes expressed in nervous tissue, calcium channel genes, the mouse homolog of the cystic fibrosis transmembrane conductance regulator gene, and various genes for transcriptional factors. Several of these genes map at or near known mouse mutations and therefore are potential candidates for these mutations. Thus, one gene specifically expressed in melanocytes was mapped near the silver locus on chromosome 10, and another gene specifically expressed in brain was mapped near the coloboma neurological mutation and shown to be deleted in mutant coloboma mice.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00353-09 LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural and Functional Studies of Mammalian Endogenous Retroviral Sequences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. S. Khan	Microbiologist	LMM, NIAID
Others:	P. Kedar	Visiting Associate	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AO 00304-11 LMM

PERIOD COVERED

October 1, 1992 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Retroviral Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dr. J Silver	Medical Officer	LMM, NIAID
Others:	Dr. T. Limjoco	Visiting Associate	LMM, NIAID
	Dr. K. Fujita	Visiting Associate	LMM, NIAID
	Dr. P. Dickie	Visiting Associate	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prior infection of cells with one retrovirus often renders them resistant to "super-infection" with another retrovirus. The resistance is thought to arise because viral envelope protein produced in the infected cell interacts with receptor, rendering it incapable of intereacting normally with incoming viruses. A similar mechanism may be responsible for certain forms of inherited resistance to retroviruses in animal systems in which the resistance is co-inherited with an expressed, "endogenous" retroviral envelope gene. One such endogenous retroviral envelope gene in mice genetically linked to a resistance trait (Fv-4) was cloned in LMM several years ago. To investigate the in vivo effects of this gene, we generated transgenic mice in which the Fv-4 envelope gene is driven by its associated flanking cellular promoter. Two transgenic strains were obtained. Southern blot analysis and genetic breeding experiments showed that each strain contains a transgene in a single (and distinct) location in the mouse genome. Northern blot analysis showed that the transgenes are expressed to high level in relevant lymphoid tissues. Susceptibility to retroviral infection In vivo was assayed by inoculating mice with Friend virus and scoring splenomegaly and spleen virus titer at various times after infection. Both transgenic mouse strains were markedly resistant to infection as assayed by XC test, although at high doses of inoculum some transgenic mice developed splenomegaly, indicating that the resistance was not absolute. This model of natural resistance to retroviruses could form the basis of "gene therapy" approaches to HIV. We are investigating the effects of placing other potential retroviral resistance genes, including the HIV envelope, downstream of the FV-4 promoter in tissue culture and transgenic systems.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00415-08-LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. A. Martin	Chief	LMM, NIAID
Others:	K. Strebel	Senior Staff Fellow	LMM, NIAID
	F. Maldarelli	Med.Staff Fellow	LMM, NIAID
	L-J Chang	Visiting Associate	LMM, NIAID
	K. Peden	Expert	LMM, NIAID
	R. Willey	Biologist	LMM, NIAID
	P. Mounts	IPA	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The HIV-encoded Vpu protein is an integral membrane phosphoprotein which is present in high concentrations in virus-producing cells but appears to be excluded from virus particles. Vpu forms homopolymeric complexes *in vivo* and *in vitro*; protease protection experiments indicate that Vpu possesses the topology of a Class I integral membrane protein but lacks the signal peptide processing signal. An analysis of potential interactions of Vpu with other viral or cellular proteins demonstrated that it affected the processing of the HIV envelope glycoprotein precursor gp160 and the intracellular stability of CD4. In the absence of Vpu, gp160 and CD4 form stable intracellular complexes which cannot be transported intracellularly. In the presence of Vpu, gp160/CD4 complexes are destabilized and normal synthesis of gp120/gp41 ensues. A series of pulse/chase experiments, designed to investigate the mechanism of the Vpu-dependent destabilization of gp160/CD4 complexes revealed that Vpu induces the rapid degradation of CD4 reducing its half-life from normally 6 hours to 12 minutes. Degradation was shown to depend on the retention of CD4 in the endoplasmic reticulum of the cells which is accomplished by the formation of gp160/CD4 complexes. However, gp160 is not directly involved in the degradation of CD4 and its function as a CD4 trap can be substituted by Brefeldin A, a drug that blocks membrane traffick in the endoplasmic reticulum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZAI-00467-07-LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Polypeptides Encoded by the HIV-1 Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sundararajan Venkatesan	Medical Officer	LMM, NIAID
Others:	Yong Suk Nam	Visiting Fellow	LMM, NIAID
	Hee-Sung Park	Visiting Fellow	LMM, NIAID
	Susanne Hiller	Visiting Fellow	LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using a vaccinia virus recombinant system, we demonstrated before that self-assembly of p55 GAG protein is sufficient to form the skeletal framework of the nascent human immunodeficiency virus (HIV-1) particle. The particles which budded from the cells infected with a vaccinia-GAG construct were mostly spheres with a concentric ring of electron dense material. Expression of the GAG and POL proteins resulted in mature particles containing a condensed core which assumed the nucleoid structure characteristic of lentiviruses. When the POL frame in the GAG-POL ORF was truncated at the end of the protease domain, p55 GAG processing was markedly reduced and the maturation of the resultant particles was defective.

The mechanism of RNA encapsidation during retrovirus particle formation is still unclear. In particular, the nature and the identity of the RNA sequences and the gag subunits involved in HIV capsidation remain to be solved. We have now developed a simple *in vivo* model for HIV-1 RNA packaging. A subgenomic HIV RNA encompassing R, U5 and U3 elements surrounding the minimal packaging sequence was expressed from the T7 promoter using vaccinia T7 polymerase vector. Co-infection with GAG or GAG-POL recombinant vaccinia viruses led to HIV particles that selectively packaged the mini HIV RNA. We have expressed cleavage defective mutants of GAG using the vaccinia vector. Mutations that eliminate the myristoylation or the nucleic acid binding CYS containing motif (NBCys) of the p9 subunit of GAG protein have been analyzed in the context of GAG-POL expression to examine whether they interfere with RNA packaging. This system is being used to define the molecular parameters of RNA capsidation. It is our goal eventually to develop molecular strategies to interfere with RNA packaging and particle maturation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00527-05 LMM

PERIOD COVERED

October 31, 1992 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Cloning and Characterization of Human Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Theodore S. Theodore	Res. Microbiologist	LMM, NIAID
Others:	George Englund	Biologist	LMM, NIAID
	Malcolm A. Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Heterogeneity among AIDS retroviral genomes is a distinct feature of lentiviruses. Molecular cloning, restriction enzyme analysis, nucleotide sequencing, and polymerase chain reaction techniques were used to characterize isolates of Human Immunodeficiency Virus Type 1 (HIV-1). The objective was to obtain information pertaining to the structure and diversity of HIV with respect to its biochemical, pathogenic, and antigenic variability. Most HIV isolates we have cloned are T-cell tropic. We have succeeded in obtaining a complete molecular clone from a macrophagetropic viral isolate. Although T-cells are the major target for HIV replication in peripheral blood, macrophages represent the predominant HIV-infected cell type in most tissues. Macrophages are probably the primary reservoir of HIV, and sustain a persistent infection in individuals for many years. A single HIV macrophagetropic isolate was molecularly cloned, and upon transfection into HeLa cells followed by cocultivation on PBLs or macrophages, produced viral particles (RT). The virus produced was infectious and could replicate in either PBLs or macrophages. It was unable to replicate in a continuous T-cell line (CEM). Growth studies and biochemical analysis with this isolate are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00528-05-LMM

PERIOD COVERED

October 1, 1992 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Interactions of HIV-1 Regulatory Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Venkatesan	Medical Officer	LMM, NIAID
Others:	H. S. Park	Fogarty Fellow	LMM, NIAID
	Y. S. Nam	Fogarty Fellow	LMM, NIAID
	S. Hiller	Fogarty Fellow	LMM, NIAID
	S. Gerstberger	Microbiologist	LMM, NIAID

COOPERATING UNITS *(if any)*

S. M. Holland Medical Officer LHD, NIAID

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Trans-activation of unspliced or partially spliced HIV RNAs by the REV regulatory protein is crucial for virus replication and is dependent on the sequence specific binding to a highly structured 244 nucleotide RRE RNA in the viral env gene. REV requires the presence of a nine nucleotide 5'-CACUAUGGG-3' RNA motif that must be presented as a stem-bulge-stem structure and must contain at least 2 Gs, one of which must be unpaired. But, RRE interaction is not an absolute requirement for REV function. REV will activate HIV mRNAs containing a heterologous MS2 phage operator sequence if directed to the operator via the RNA binding motif of the MS2 phage coat protein (MS-C) as a fusion protein. Mutation in the MS operator that abolished the coat protein binding *in vitro* rendered the mutant chimera non-responsive to the fusion protein *in vivo.* These studies imply that REV binding is the primary function of RRE, and cellular factors specifically reacting with RRE may not be necessary for REV function. While REV can be tethered to the viral RNAs by another protein, the structural integrity of REV was still required for optimal trans-activation. Two positive clones, RRBFI and RRBFII, which expressed major protein species of 43 and 45 kDal were obtained. These proteins were identical to the previously reported TAR binding protein(s), TRBP.

We have extended our studies on the effect of HIV-1 NEF protein on cellular genes. HIV NEF protein decreases in the surface expression of CD4 receptor both in CD4+ T cells and non-lymphoid cells. The CD4 effect was due to accelerated recycling and degradation of the surface CD4. CD4 molecules which are retained in the ER and CD lacking the cytoplasmic domain are not regulated by NEF. Myristoylation of NEF was required for the effect on CD4. NEF effect on the CD4 in T cells may have profound effect(s) on T cell activation pathways.

HIV-1 LTR linked NEF expression in transgenic mice was confined to skin and was associated with papillomatous skin lesions that varied in severity depending on the level of NEF expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00547-03 LMM

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Human Retrovirus trans-Regulatory Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kuan-Teh Jeang	Medical Officer, Research	LMM, NIAID
Others:	A. Gatignol	Special Volunteer	LMM, NIAID
	B. Dropulic	Visiting Associate	LMM, NIAID
	Y. Chang	Staff Fellow	LMM, NIAID
	O.J. Semmes, IV	Staff Fellow	LMM, NIAID
	L. Huang	Visiting Associate	LMM, NIAID
	M. Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the mechanisms by which HIV-1 Tat and HTLV-I Tax proteins activate transcription. Recent findings relevant to the HIV-1 system include the following: 1) The Tat-responsive element in the TAR RNA has been better defined. Besides the commonly recognized bulge element, we have recently demonstrated that the stem sequences immediately flanking the bulge are also important. This finding has been independently confirmed by two other laboratories. 2) The DNA target in the LTR for Tat response has been more precisely characterized. In particular, it is increasingly clear that the Sp1 elements and the particular HIV-1 TATAA box are very important for optimal trans-activation. 3) A second cellular TAR RNA-binding protein has been isolated. This protein has been identified as the human autoantigen La.

For the HTLV-I system, we have characterized 47 different mutant Tax proteins. Results from this analysis have allowed us to define two different types of activation domains for Tax. The first is a domain necessary for interaction with the CREB/Ap1 pathway. The second is a domain involved in the NF-κB pathway. The former is presumably used for activation of the HTLV-1 LTR while the latter presumably account for Tax activation of the HIV-1 LTR. We have also designed and tested in vitro a ribozyme specific for the HIV-1 U5 sequence. Results here demonstrated that high expression of this ribozyme can functionally suppress productive HIV-1 production in CD4+ T-cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00588-02 LMM

PERIOD COVERED
October 1, 1990 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Simian Immunodeficiency Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Khan	Microbiologist	LMM, NIAID
Others:	T. Galvin	Guest Researcher	LMM, NIAID

COOPERATING UNITS *(if any)*

L. Lowenstine	Assoc. Professor	U. of Calif., Davis

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	.6	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Terminated

PHS 6040 (Rev. 5/92)

LABORATORY OF MALARIA RESEARCH
1992 Annual Report
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Laboratory of Malaria Research
National Institute of Allergy and Infectious Disease
Summary - October 1, 1991 - September 30, 1992

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

The Laboratory of Malaria Research exists for the first time as a separate unit devoted to using basic research to solve the problems of malaria. Despite our independence from the Laboratory of Parasitic Diseases, we still interact closely with some common secretarial support, common mail distribution, a common equipment room, a joint weekly seminar and shared insectary facilities. The clinical service is primarily the responsibility of LPD, but Tom Wellems in LMR is the coordinator for physicians in LMR who work in the clinical program. We have plans to use the clinical service for vaccine trials. The creation of a new laboratory will give us more flexibility to develop programs aimed at alleviating the severe problems of malaria, exacerbated most recently by chloroquine resistance.

This year has been most rewarding in the area of mosquito vector biology, an area of research that has come into its own after years of nurturing. The scientific accomplishments have been many and important. A. Warburg and L.H. Miller have completed the *in vitro* culture of the mosquito stages of the malaria parasite. This was the only part of the cycle that had not been previously grown in culture. The success in *P. gallinaceum* depended on the use of basement membrane matrix (matrigel) and co-cultivation with a cell line. Growth of *P. falciparum* had the additional problem of converting zygotes to ookinetes and then into oocysts and sporozoite.

Another area was built on the work of a previous member of the laboratory, R. Rosenberg, who showed that invasion of salivary glands by sporozoite is receptor-specific. To identify the receptors, it was necessary to first develop ways to study the interaction of sporozoite with salivary glands under controlled conditions. M. Touray, Warburg and Miller have discovered that midgut sporozoite and salivary gland sporozoite are different in that midgut sporozoite invade salivary glands; sporozoite from the salivary glands cannot reinvade salivary glands. Midgut sporozoite are now being used to identify the receptors on salivary glands with the goal of modifying the glands to make them refractory to invasion.

M. Shahabuddin and D. Kaslow have studied the role of the parasite chitinase previously identified by M. Huber and L.H. Miller. They found that the parasite could not penetrate the peritrophic membrane in the mosquito midgut in the presence of the chitinase inhibitor, allosamidin. Because addition of allosamidin-resistant chitinase abrogated the transmission-blocking activity of allosamidin, the blocking effect of allosamidin appears to be chitinase-specific. Parasite chitinase is now a potential target for a transmission-blocking vaccine.

Introduction of foreign genes into the mosquito germ line has been performed at low frequency. Experiments with effects of foreign genes on malaria parasites have not been possible. E. Miahle and L.H. Miller have successfully obtained transient expression of the luciferase gene, using biolistics in very young embryos. The method is now being tested for stable integration info germ line.

The Mali project in West Africa has now been in place for three years with the goal of developing the Malian scientists to study the major vector in the world, *Anopheles gambiae*, in a setting where American scientists can productively interact. The official opening of the center occurs this year. Three projects, which are in the early phase, are underway. Microbiology of larvae is in collaboration with R. Colwell and D. Bryant, University of Maryland, and studies of chloroquine resistance begun by D. Krogstad during his sabbatical at the NIH are to be continued through Tulane. The third project, population studies done by K. Mathiopoulos, G. Lanzaro and T. McCutchan has focused on the development of tools to separate mosquitoes of the *An. gambiae* complex and to follow gene flow in the population.

D. Kaslow has led a most productive program toward the first clinical trials of a transmission-blocking vaccine. After the cloning of the gene encoding are the targets of transmission-blocking antibody, a collaboration with Chiron has led to the production of a yeast-derived recombinant Pfs25 that, when adsorbed to alum, leads to complete blocking of transmission. To eliminate the requirement for repeated injections, Pfs 25 has been formulated as a slow release particle in a biodegradable polymer which has been shown to induce complete transmission-blocking activity and high titer antibodies that persist for more than 300 days after a single immunization. Much work has gone into the development of Pfs 25 as a GMP product for clinical trials.

Side-by-side with the development of Pfs25 has been the search for other potential targets for transmission-blocking immunity by D. Kaslow and his colleagues. Shahabuddin's work on chitinase is mentioned above. K. Williamson has cloned the gene encoding the 230 kD transmission-blocking target antigen. The gene was cloned by oligonucleotide probes derived from microsequence of the affinity- purified protein. The protein is encoded by a gene of 12.5 kb, 9.4 kb of which has been sequenced. Antisera to one fusion protein derived from the gene immunoprecipitates the native protein and reacts with the surface of gametes. The challenge with this large protein is to make recombinant immunogens that will have transmission-blocking activity.

P. Duffy in D. Kaslow's laboratory has isolated a 28 kD surface protein of *P. gallinaceum* ookinetes that induces complete transmission-blocking immunity. It blocks the development of zygotes to ookinetes and probably also blocks invasion of the midgut. The gene encoding this protein has been cloned and found to be homologous to Pgs25. Both consist of four EGF-like domains. Because EGF domains are cysteine rich, the target epitopes are conformation dependent as antisera raised against reduced Pgs28 do not block transmission. An analogous gene has been identified in *P. falciparum*.

In order for a vaccine to be placed in the field, it must contain components against *P. vivax* as well as *P. falciparum*. Genomic probes from known transmission-blocking proteins of

P. falciparum do not cross-hybridize. As gametes of *P. vivax* are unobtainable, M. Fried and D. Kaslow have obtained gametes of *P. knowlesi* and have developed methods to transform them into ookinetes. Monoclonal antibodies to sexual stage parasite antigens are currently being developed for protein purification and cloning of the gene. From these genes of *P. knowlesi*, it should be possible to clone comparable genes of *P. vivax* from genomic DNA.

One of the most demanding tasks in transmission-blocking vaccine development is the consistent production of highly infectious gametocytes for membrane-feeding assays. A method is being developed by D. Keister for cryopreservation of gametes to maintain a fozen supply that can be thawed as needed.

T. Wellems identified the genomic region responsible for chloroquine resistance, and much work is directed towards cloning this gene. He has, in the last year, narrowed the region to 200 kb on the parasite's seventh chromosome. In collaboration with Ravetch at Sloan Kettering Hospital, yeast artificial chromosomes (YAKs) that span the region have been constructed and mapped in detail. 10 kb have been sequenced and further work on cloning cDNA from this region is planned by V. Heatwole. When candidate genes from the region are identified, transfection will be needed to prove that they encode the chloroquine-resistant gene. As transfection has not yet been accomplished in malaria, a major effort has been expended in this direction. First, D. Krogstad and T. Wellems have made a construct for homologous recombination in *P. falciparum*. The construct was of a dihydrofolate reductase-thymidilate synthetase fusion gene from a pyrimethamine resistant parasite. It was highly unstable until the 3' noncoding region was removed and now will be introduced into *P. falciparum* and *P. gallinaceum* by biolistics. D. Peterson is attempting to make a construct from *P. gallinaceum* for introduction by electroporation into *gallinaceum* sporozoite.

The gene encoding the S-adenosylhomocystein hydrolase was cloned by C. Kreedon and T. Wellems for study of the active site for targeted drug development.

Over the past year, T. McCutchan's laboratory has devoted much effort to the isolation and characterization of mutants of *Plasmodium*. Of particular interest has been auxotrophic mutants whose variation from wild type can be metabolically defined. G. McConkey and T. McCutchan have irradiated a cloned line of *P. falciparum* in a fashion that should predominantly produce a small number of deletions in the DNA of the recipients and have used a selection procedure to isolate mutants of the parasite that are dependent for growth upon exogenous sources of P-aminobenzoic acid (PABA). They isolated four cloned lines that require PABA. All are comparable with the parent strain with regard to PABA uptake, but, unlike the parent clone, two can not synthesize PABA *de novo*, as shown by HPLC of metabolically labelled intermediates. The requirement for exogenous PABA in the other two remains unresolved. This substantiated indications that a PABA synthetic pathway exists in the parasite. It also should provide an interesting system to study parasite growth and host response to the presence of parasites under conditions where the growth of the parasite can be regulated by controlling the availability of a nutrient.

McConkey and T. MuCutchan have also isolated a new class of drug-resistant mutants of *P. berghei.* One set of mutants is resistant to pyrimethamine while another is resistant to 5-fluoroorotic acid (5-FOA), drugs which interfere with *de novo* pyrimidine synthesis. Both appear to grow normally when they are given supplemental pyrimidines. The mutations were generated by irradiation of the *P. berghei* ANKA clone as described above for *P. falciparum.* A mechanism whereby *Plasmodium* may become resistant to pyrimethamine and 5-FOA is by elimination of pyrimidine synthesis and reliance on a salvage pathway. Pyrimidine uptake experiments indeed indicate that the drug-resistant mutants can now salvage pyrimidines, a pathway not previously detectable in parasites.

The research towards identification of new immunogens for asexual vaccines continues to explore unmapped areas in vaccinology with the focus on Salmonella as vector. S. Kumar and L.H. Miller have completed their first monkey trial with MSP-1 recombinant in Salmonella. This was the first demonstration that attenuated Salmonella will infect monkeys, that the vaccine induced antibodies and that the vaccination delayed onset of parasitemia, but then did not control infection. The parasites in the immunized animals were from the same clone as the parasites inoculated.

Study of invasion has made two discoveries. First, J. Adams and L.H. Miller showed that the Duffy binding molecule is a member of a family that includes the sialic acid binding protein of *P. falciparum.* Second, G. Ward, J. Dvorak and L.H. Miller demonstrated that the parasitophorous membrane derives from the erythrocyte membrane. This includes the inner leaflet of the bilayer which, in a recent paper, was claimed to not enter the vacuole.

Special recognition during the year went to R.W. Gwadz who was named Honorary Fellow of the Hebrew University of Jerusalem, Honorary Member of the Board of Directors of the Ain Shams University (Cairo), Honorary Fellow of the Egyptian Society of Parasitologists and the 1992 PHS Outstanding Service Medal.

L.H. Miller was induced into the Institute of Medicine and was the major lecturer at the 1991 ICAAC meeting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00108-21 LMR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Studies on the Immunobiology of Malaria

PRINCIPAL INVESTIGATOR	L. H. Miller	Chief	LMR, NIAID
	S. Kumar	Visiting Associate	LMR, NIAID
Others:	T. McCutchan	Senior Scientist	LMR, NIAID
	T. Jing-Hui	Visiting Fellow	LMR, NIAID
	J. Gorden	Biologist	LMR, NIAID
	D. Kaslow	Senior Staff Fellow	LMR, NIAID

COOPERATING UNITS *(if any)*
J.A. Berzofsky, Sr. Scientist, Metabolism Branch, NCI; R. Houghten, M. So, and F. Heffron, Univ. of Oregon, Portland; M.F. Good, QIMR, Brisbane, Australia; Arba Agar, U. of Miami; William E. Collins, CDC, Atlanta, GA

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Immunity to asexual erythrocytic parasites in certain rodent malarias is dependent on cell mediated mechanisms that are dependent on CD4+ T cells and the spleen and are independent of antibody. Such immunity can be induced by Salmonella and malarial antigens. The present studies are designed to identify those proteins and T cell epitopes that will lead to protection. In addition, we are developing studies to test constructs in Salmonella and BCG that will lead to protection against *Plasmodium falciparum* in *Aotus* monkeys.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00208-12 LMR

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas F. McCutchan	Microbiologist
Others:	G. McConkey	Senior Staff Fellow
	Y. Lee-Wickner	Technician
	Jun Li	Guest Worker

COOPERATING UNITS *(if any)*

None

LAB/BRANCH Laboratory of Malaria Research

SECTION Molecular Biology Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Molecular Biology Section is involved in producing attenuated lines of human and rodent malarias which should prove useful in the study of both the biochemistry of parasites and the development of immunity to parasites. We have developed auxotrophic mutants of both species of parasite. We are also studying the complementation of yeast mutants with genes derived from malaria parasites in an effort designed to characterize genes involved in development and sexual differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER
Z01 AI 00240-11LMR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR	D.C. Kaslow	Senior Staff Fellow	LMR, NIAID
Others:	D. Rawlings	Guest Researcher	LMR, NIAID
	K. Williamson	Guest Researcher	LMR, NIAID
	M. Shahabuddin	Visiting Fellow	LMR, NIAID
	M. Fried	Visiting Fellow	LMR, NIAID
	D. Keister	Biologist	LMR, NIAID
	O. Murtova	Special Volunteer	LMR, NIAID
	R. Hearn	Entomologist	LMR, NIAID
	M. Criscio	Student Trainee Biol	LMR, NIAID

COOPERATING UNITS *(If any)*
J. Coligan, Branch Chief, BRB, NIAID, S. Isaacs, Medical Staff Fellow, LVD, NIAID; B. Moss, Lab Chief, LVD, NIAID; J. Shiloach, Unit Chief, LCBD, NIDDK; Chiron Corporation, Emeryville, CA (P. Barr and I. Bathurst); Weth Laboratories, Padnor, PA (P. Hung); Catholic U,

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	4.3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

COOPERATING UNITS *(If any)* (Continued)
Nijmegen, The Netherlands (J. Schoenmakers & T. Ponnudurai); Pasteur Institut, Paris, France (G. Langsley); Scripps Clin. Res. Fnd, La Jolla, CA (A. Satterwaith); CDC, Atlanta, GA (B. Collins); US Army (Walter Reed Army Institute of Research, Washington, DC - R. Ballou & J. Sadoff; Ft. Dietrick, MD - J. Dalrymple); Case Western Reserve School of Medicine, Cleveland, OH (M. Aikawa), Virogenetics Corp., Troy, NY (E. Paoletti), Smith, Kline & Beechman, King of Prussia, PA (M. Gross).

SUMMARY OF WORK

Four potential target antigens (Pfs25/Pgs25, Pfs40, Pfs230, and Pgs28) of transmission-blocking antibodies have now been cloned in our laboratory, and a fifth (chitinase) has been identified. Of the four target antigens that have been cloned, all have been expressed in one or more recombinant expression systems, but only recombinant Pfs25 has induced transmission-blocking antibodies in laboratory animals. Our immediate goals are to 1) test in humans the safety, immunogenicity, and efficacy of a Pfs25 subunit vaccine and design a means of testing the efficacy of such a transmission-blocking vaccine in the field; 2) determine the role, if any, that the calcium-binding Pfs40 plays in sexual development and ascertain if Pfs40 is a target of transmission-blocking antibodies; 3) identify and express as recombinant protein the B-cell epitopes in Pfs230 responsible for the transmission-blocking activity of our monoclonal antibodies to Pfs230; 4) isolate the gene from *P. falciparum* encoding the protein analogous to the *P. gallinaceum* Pgs28; 5) determine if antibodies to chitinase completely block transmission, and if so, isolate and express the gene encoding this ookinete enzyme; and 6) isolate analogous genes (if they exist) to these five proteins from the other human malaria parasites. Our more long term goals include identifying new target antigens on sexual stage parasites, and defining the molecular mechanisms involved in fertilization of malarial parasites.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 AI 00241-11 LMR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR	L. H. Miller	Chief	LMR, NIAID
Others:	C. Plowe	Staff Fellow	LMR, NIAID
	D. Hudson	Microbiologist	LMR, NIAID
	C. Chitnis	Visiting Fellow	LMR, NIAID
	D. Kaslow	Sr. Staff Fellow	LMR, NIAID
	G. Ward	Visiting Associate	LMR, NIAID
	S. Dolan	Medical Staff Fellow	LMR, NIAID

COOPERATING UNITS *(if any)*
WRAIR, Washington, DC (F. Klotz and J.D. Haynes); Case Western Reserve University, Cleveland, OH (M. Aikawa)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	4.3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage upon which immunity may work to block invasion. Thus, merozoite surface components are of interest because of their role in erythrocyte recognition and as antigens for induction of protective immunity. We are identifying *P. knowlesi, P. vivax* and *P. falciparum* receptors for attachment to monkey and human erythrocytes. The components in the junction and the signaling after merozoites make contact with erythrocytes is also under study.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00248-11 LMR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR	R. W. Gwadz	Section Head	LMR, NIAID
	L. H. Miller	Laboratory Chief	LMR, NIAID
Others:	R. K. Sakai	Senior Scientist	LMR, NIAID
	K. Vernick	Senior Staff Fellow	LMR, NIAID
	D. Kaslow	Medical Staff Fellow	LMR, NIAID
	M. Shahabuddin	Visiting Fellow	LMR, NIAID
See below	A. Warburg	Visiting Associate	LMR, NIAID

COOPERATING UNITS *(if any)*
National Medical School, Bamako, Mali (Dr. Y. Toure); Red Cross Research Center (Dr. R. Williams)

LAB/BRANCH
Laboratory of Malaria Research

SECTION
Medical Entomology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
5.7	3.9	1.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Others (Cont.):
M. G. Touray, Visiting Fellow, LMR, NIAID; E. Mialhe, Guest Researcher, LMR, NIAID; D. Seeley, Supervisory Entomologist, LMR, NIAID; P. Pimenta, Guest Researcher, LPD, NIAID; H. G. Coon, Senior Scientist, LG, NCI; K. Mizuuchi, Senior Scientist, LMB, NIDDK; M. S. Lee, Guest Researcher, LMB, NIDDK; J. Kassis, Senior Scientist, DBB, CBER

The biology of anopheline mosquitoes is being studied in relation to the capacity of these vectors to transmit malaria. Genetic, molecular, biochemical and immunological studies are seeking to describe mechanisms in the mosquito which enhance or retard development of the malaria parasite with emphasis on the behavior of ookinetes, oocysts and sporozoites.

To facilitate these studies: 1) techniques are being developed to cultivate the sporogonic (mosquito) stages of the malaria parasite *in vitro*, 2) methods are being improved for cloning and transposing genes into mosquito germ lines, and 3) systems are being evaluated for the cryopreservation of mosquito embryos to increase our capacity to store genetic material.

The ability to identify, clone, and transpose genes which regulate mechanisms which render mosquitoes refractory to parasite development should facilitate development of control strategies based on the introduction into the field of mosquito populations incapable of transmitting malaria.

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U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00483-07 LMR

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Malaria Genetics and Pharmacology

PRINCIPAL INVESTIGATOR	T. E. Wellems	Section Head	GPS, LMR, NIAID
Others:	S. Dolan	Medical Staff Fellow	GPS, LMR, NIAID
	D. Peterson	Senior Staff Fellow	GPS, LMR, NIAID
	K. Creedon	Guest Worker	GPS, LMR, NIAID
	J. Herrfeldt	Technician	GPS, LMR, NIAID
	D. Krogstad	Visiting Scientist	Tulane School of Medicine
	F. Gyang	Fulbright Scholar	University of Ghana

COOPERATING UNITS *(if any)*
Sloan Kettering Institute, New York (Dr. J. Ravetch); Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. (W.K. Milhous); Catholic University, Washington, D.C. (P. Rathod).

LAB/BRANCH
Laboratory of Malaria Research

SECTION
Genetics and Pharmacology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Genetic basis of chloroquine resistance. Linkage analysis indicates that chloroquine resistance in *P. falciparum* is controlled by a single gene or closely linked group of genes within a ≈200 kilobase segment on the parasite's seventh chromosome. We are using yeast artificial chromosomes to map this segment in detail and are identifying transcribed segments that should lead to a candidate resistance gene.

Other drug development targets. Studies have progressed on two *Plasmodium* enzymes: dihydrofolate reductase-thymidylate synthase (DHFR-TS) and S-adenosylhomocysteine hydrolase (AHCY). Knowledge of point mutations in DHFR-TS is being used as a basis for epidemiological surveys and for development of alternative drugs. The predicted amino acid sequence of *P. falciparum* AHCY is now determined. Heterologous expression and purification of the enzyme is being pursued for kinetic and functional comparisons with mammalian AHCY.

Transfection of *Plasmodium.* Efforts are focused on development of constructs and on DNA delivery methods for parasite transfection. Stable transfection is being attempted with a dihydrofolate reductase-thymidylate synthase (DHFR-TS) construct. Transient expression experiments are being pursued with luciferase constructs. Ballistic particles and electroporation are being evaluated for DNA delivery.

Genetic analysis of red cell invasion. A switch to an alternative pathway of red cell invasion was detected in a *P. falciparum* clone made to propagate in neuraminidase-treated, sialic acid-deficient red blood cells. The switch may involve a mechanism in which certain genes are activated or rearranged. Differential and subtractive screening methods have so far been unsuccessful. An alternative approach using genetic linkage analysis is being examined.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-884-638

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00629-01 LMR

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Population Biology of *Anopheles gambiae* in Africa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	T. F. McCutchan	Microbiologist
	L.M. Miller	Head, Malaria Lab
	R.W. Gwadz	Health Officer
Others:	R.K. Sakai	Expert (Mali)
	G.C. Lanzaro	Guest Worker

See Below

COOPERATING UNITS *(if any)* National School of Medicine, Bamako, Mali (Dr. Y.T. Toure); Inst. of Parasitology, Univ. of Rome (Dr. Mario Couzzi); Univ. of Maryland Biotechnology Institute (Dr. R. Colewell)

LAB/BRANCH
Laboratory of Malaria Research

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

K. Mathiopoulos	Fogarty Fellow
J. Li	Guest Worker
D. Bryant	Guest Worker

An. gambiae s.s. is known to be highly variable in terms of chromosomal polymorphisms which are related to chromosomal inversions. It has been suggested that these inversions are adaptive, allowing the species to occupy a variety of habitats and thrive. It is the highly adaptive nature of this species which makes it a powerful vector of human malaria. Our goal is to undertake an in-depth study of the genetic structure of *An. gambiae s.s.* and, in addition, to look for possible correlations between specific genetic polymorphisms and vectorial capacity for malaria. We will approach questions such as 1) How much gene flow or gene exchange is there among geographic populations and between individuals carrying different inversions? 2) How is genetic polymorphism distributed within the genome? 3) Is there a correlation between vectorial capacity and specific karyotypes?

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1992 Annual Report
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Annual Report
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1991, to September 30, 1992

RESEARCH HIGHLIGHTS

LMSF scientists study disease-causing microbes and their interactions with eukaryotic hosts. General goals are to explore the infectious strategies and mechanisms employed by pathogenic microorganisms and the host responses that might be exploited for preventing or treating infections. Special attention is paid to genetic events that generate microbial variations, especially as they relate to avoidance and/or evasion of host defenses.

During FY 1992, LMSF staff members studied assorted aspects of *Neisseria gonorrhoeae* (Gc), *Borrelia burgdorferi* (Bb), group B streptococci (GBS), *Pseudomonas aeruginosa*, and human immunodeficiency virus (HIV). Other LMSF research utilizes more defined systems to explore more basic aspects of parasitic processes (including interspecies genetic transfer), microbial mutation in response to hostile environments, replication of unusual forms of genomic DNA (such as linear plasmids), and molecular aspects of host defenses such as defensins. Utility of antibodies conjugated to ricin or other toxins and directed toward surface-exposed moieties of selected viral and bacterial pathogens was also explored.

Neisseria gonorrhoeae: Earlier studies in LMSF established that virulence of Gc depends on their having pili, enabling them to avidly attach to mucosal cells; these organisms display remarkable abilities to vary their pili *in vitro* and *in vivo*. Stuart Hill has explored the contributions of DNA-transformation and intragenomic recombination to pilin subunit variation. His studies suggest that DNA-transformation has little relevance to pilus antigenic variation. Hill has also defined 'bending' of DNA in the -35 control region of pilE, consistent with its enhancing *pil*E transcription and, consequently, its mutability. Other Gc components including colony opacity-associated outer membrane proteins (Opa), have implied but unproven pathogenetic functions. John Swanson has been exploring these outer membrane constituents as to their influence on surface charge of Gc. He finds that Gc expressing particular Opa proteins can attain a 'capsule' comprised of polyanions (DNA, RNA, sulfated polysaccharides) and this markedly diminishes their adherence to epithelial cells and renders them 'less-visible' to polymorphonuclear leukocytes *in vitro*. Bob Belland has extended his studies on the role of *opa* structure to its expression and phase-switching. He finds that the ability of the leader peptide-encoding sequence of each *opa* to form an intramolecular triple-stranded structure influences the frequency at which it switches on/off. Expression of certain Opa proteins endows gonococci with ability to adhere to epithelial cells and neutrophils. Belland and Tie Chen corroborated their earlier findings with β-lactamase::*opa* operon fusions to demonstrate that *E. coli* carrying such fusions behave like gonococci that express the same Opa. Belland has recently identified an operon from gonococci that contains genes encoding novel (Type III) restriction-modification enzymes as well as recently cloning an *ihf* analogue as well as *gyr*A and *gyr*B genes of Gc, all of which have potential relevance to changes in *opa* expression. Chen has demonstrated that only OpaA+ gonococci adhere to and are ingested by tissue culture cells when assayed as pilus$^-$ phenotypes. He is also exploring the role of localized polymerization of actin in host cells that interact with gonococci. Steven Fischer has identified a peptide ('fourth loop') common to all Opa proteins that appears to be involved in mediating gonococcal interactions *in vitro* with polymorphonuclear leukocytes. He has also found that OpaA$^+$ gonococci adhere better to laminin, vitronectin, and fibronectin than Opa- and other Opa$^+$ organisms.

Borrelia burgdorferi: *Borrelia burgdorferi* (Bb), the causative agent of Lyme disease, presents many pathogenetic unknowns regarding its acute infections and their complications. A key question with potential diagnostic and pathogenetic importance is whether Bb can vary its surface components. Patti Rosa has defined a number of sequence changes in the Bb genes encoding two major outer membrane proteins (OspA, OspB); some of these mutations lead to premature translation terminations in one or the other gene while others yield mosaic OspA/OspB molecules. Neil Margolis has found 'null phenotypes' relative to OspA and OspB that have no coding sequence alterations in *osp*A or *osp*B; transcriptional 'control' is suspected to be responsible. These findings raise provocative questions as to the potential usefulness of vaccines and diagnostic reagents based on OspA and OspB. In collaboration with Tom Schwan (LVP), Rosa is exploring the relationships between these *osp* rearrangements and virulence of Bb. An intriguing question of potential pathogenetic import is how Bb survives and multiplies in both a tick vector (at ambient temperatures) and in mammalian hosts (at 37°C). Kit Tilly has identified and molecularly cloned the Bb homologues of *dnaK*, *dnaJ* and *grpE* which all represent 'heat-shock' genes in *E. coli* and are likely involved in growth of Bb at differing temperatures like those in host versus tick vector. She is also exploring methods for genetic transfer into Bb.

Immunoglobulin biology: Group B streptococci (GBS) remain important causative agents of neonatal sepsis, but little is known about the factors that dictate virulence and pathogenicity of these organisms. Seth Pincus has identified colony opacity variants of GBS that differ in several ways including: chain formation, cell wall morphology, antibody binding, immunogenicity, complement activation, phagocytosis, and virulence in experimental animals. These variants switch from one to another by unknown mechanisms. Pincus previously designed and constructed immunotoxins that conjugate ricin A chain to monoclonal immunoglobulin molecules of desired specificities as potential agents for treating HIV infections; several directed against HIV envelope components were highly effective in eradicating HIV infection of tissue culture cells except when variant viruses arose. These variants have been molecularly analyzed by Pincus to better direct design of more effective immunotoxins. Recently he purified anti-gp160 polyclonal antibodies from sera of HIV-positive individuals and found that these were highly effective in eradicating HIV infections *in vitro* when conjugated to ricin A chain. He has also found that addition of soluble CD4 markedly enhances the efficacy of anti-gp41 immunotoxins, but not those directed against gp-120. Pincus is analyzing the serum antibody responses of NIH (Bethesda) laboratory workers who were accidentally infected with a well-characterized HIV and those of individuals who have received HIV vaccines so as to precisely define those viral envelope epitopes to which antibodies are developed with time. Pincus has constructed appropriate immunotoxins against MuLV and, in collaboration with Leonard Evans (LPVD), has found that these reagents can kill viral-infected cells *in vivo*. Carol Horgan has examined structure-function relationships of immunoglobulins by constructing recombinant genes that encode antibodies with desired antigenic specificities and altered hinge regions. These recombinant molecules have proven useful for evaluating the influence of antigen-binding portion on the ability of an antibody to fix complement and to better define complement binding by antibodies.

Molecular aspects of microbial sex: Jack Heinemann has demonstrated that yeast can be 'induced' to mutate in ways that specifically allow their survival when starved for particular amino acids; the mutation that allows their avoiding such starvation occurred 10,000 - 1,100,000 times more often with selective conditions compared to those that are non-selective. The occurrence of such 'Cairnsian' events indicates that organisms have mutation propensities that transcend orthodox spontaneous mutations.

Synthetic Defensin and other gene construction: Defensins are small polypeptides that are produced by mammals, particularly in professional phagocytic cells, and have potent antibacterial properties. Kolari Bhat has assembled a totally synthetic human defensin-encoding gene from oligonucleotides synthesized *in vitro*. This involves a new strategy that he devised for assembling synthetic genes, as initially employed for *lacZ* assembly. Bhat is constructing cDNA clones from mouse macrophage RNA to identify genes that encode other antibacterial peptides.

Iron-binding by pseudomonas: Bob Ankenbauer has molecularly cloned the gene that encodes a ≈75 kDA outer membrane protein; this is a receptor for the siderophore pyochelin that is used by this organism to capture iron from its environment. He has also identified a new siderophore for these bacteria. These represent important steps toward Ankenbauer's aim to comprehensively define biosynthetic and transport components for iron acquisition by pseudomonas.

ADMINISTRATIVE REPORT

Personnel changes in LMSF during FY'92 include the departure of Ernst Kamanga-Sollo to University of Minnesota, Minneapolis; Stuart Hill to Institute of Molecular Biology, University of Oregon, Eugene; and Carol Horgan to University of Auckland, New Zealand. Hua Fang (Beijing Medical University, China) was recruited as a Visiting Fellow. Wai Mun Huang and Sherwood Casjens, University of Utah Medical Center, Salt Lake City, were Special Volunteers for three months, and Kathleen Haines, New York University Medical Center, and Emil Gotschlich, Rockefeller University, New York, were Special Volunteers for six weeks in LMSF. Vladimir Tolstikov (Institute of New Antibiotics, Moscow, Russia) spent four months at LMSF collaborating on anti-HIV immunotoxin research. Summer students included Sara Rudolph (Montana State University), Dylan Goheen (Princeton), and Hai Quan (Indiana University). A national/ international conference on The Interplay of Microbes and Their Hosts was held at RML and hosted by LMSF. Seminars were given by a number of outside guests: Stephen Barthold (Yale), Gary Peltz (Syntex), Franklin Stahl (University of Oregon), Mark Achtman (Max-Planck Institut für Molekular Genetik, Berlin), John Carlson (Princeton), Ronald Porter (Pennsylvania State University), Otto Landman (Georgetown), David Hagen (University of Oregon), Sven Bergström (University of Umea, Sweden), and Sam Coleman (University of Oregon).

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson -	Infection and Immunity
S. Pincus -	Medical advisory board for Arthritis Today

Manuscripts were reviewed by LMSF staff for the following journals: Canadian Journal of Microbiology, Cell, Infection and Immunity, Journal of Bacteriology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of Experimental Medicine, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Journal of Rheumatology, Microbial Pathogenesis, Molecular Microbiology, and Proceedings of the National Academy of Sciences USA.

Professional Posts:

S. Pincus -	Adjunct Associate Professor of Internal Medicine, University of Utah, Salt Lake City, Utah
J. Swanson -	Advisor of Extramural NIAID-sponsored Sexually Transmitted Diseases Cooperative Research Center, University of Texas Health Science Center San Antonio, Texas NIAID-MIDC Task Force on Microbiology and Infectious Diseases

Invited Lectures and Participation in Meetings and Symposia:

R. Belland -	International Conference on Genome Plasticity, Cancun, Mexico Fallen Leaf Lake Conference on Bacterial Virulence Mechanisms, South Lake Tahoe, CA The Interplay of Microbes and Their Hosts, Hamilton, MT
K. Bhat -	Science Innovation-1992, San Francisco, CA
T. Chen -	American Society for Microbiology Annual Meeting, New Orleans, LA
J. Heinemann -	National Institute of Child Health and Human Development, Bethesda, MD The Interplay of Microbes and Their Hosts, Hamilton, MT
C. Horgan -	2nd International Conference on Human Antibodies and Hybridomas, Cambridge, United Kingdom American College of Rheumatology, Boston, MA University of New Mexico, Albuquerque, NM Montana State University, Bozeman, MT
S. Pincus -	AIDS Vaccine Development Meeting, Marco Island, FL Workshop of Intramural AIDS Targeted Antiviral Program, Bethesda, MD Frontier in HIV Therapy Meeting, San Diego, CA Principal Organizer, The Interplay of Microbes and Their Hosts, Hamilton, MT Laboratory of Tumor Cell Biology, NCI, NIAID, Bethesda, MD Johns Hopkins University, Baltimore, MD Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA University of Cincinnati/Cincinnati Children's Hospital, Cincinnati, OH University of Montana, Missoula, MT
P. Rosa -	Molecular Biology of Spirochetes, Annecy, France Vth International Conference on Lyme Borreliosis (Program Committee), Arlington, VA 1st International Conference on Tick Borne Pathogens at the Host Vector Interface, St. Paul, MN Co-organizer, The Interplay of Microbes and Their Hosts, Hamilton, MT University of Montana, Missoula, MT
J. Swanson -	Graduate Division of Biomedical and Biological Sciences, Emory University, Atlanta, GA Co-organizer, The Interplay of Microbes and Their Hosts, Hamilton, MT Fallen Leaf Lake Conference on Bacterial Virulence Mechanisms, South Lake Tahoe, CA
K. Tilly -	Vth International Conference on Lyme Borreliosis, Arlington, VA

Other Activities:

J. Swanson -	Reviewed research grants for National Science Foundation, Washington, DC Consulted for Sexually Transmitted Diseases Branch, NIAID, Bethesda, MD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00193-13 LMSF

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. Swanson	Chief	LMSF, NIAID
Others:	S. A. Hill	Staff Fellow	LMSF, NIAID
	S. G. Morrison	Microbiologist	LMSF, NIAID
	J. M. Wilson	Biologist	LMSF, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	1.8	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on gonococcal surface components during the past year focused on mechanisms by which these bacterial pathogens alter the structure of their pili and on the affects that lipooligosaccharide (LOS) and opacity-associated (Opa) outer membrane protein constituents exert on gonococcal surface properties. Major recent findings include demonstration that pilin changes that follow transformation with chromosomal DNA containing pilE (expressed pilin structural gene) fused with a CAT marker result in wholesale replacement of recipient pilE with donor pilE::CAT sequence but little attendant pilin variation. When plasmids with selected pilE::CAT constructions were used for transformation donor DNA, it was clear that 5' and 3' flanking sequence homologies were necessary for complete pilE replacement. If 3' flanking homology was absent, several kinds of change in recipient pilE occurred: micro-recombination tracts, insertion of new hypervariable segments, and deletion between direct repeats of pilE. These data cast serious doubt on the importance of DNA-transformation as a mediator of pilin variation of gonococci. Gonococcal surface properties are being explored in several ways, including use of Doppler electrophoretic light scattering technology to net cell charge. Both LOS and Opa constitution influence cell charge. The charges imparted by members of the repertoire of 10+ Opa proteins expressed by a single strain has been corroborated by examining E. coli that express the analogous but recombinant genes. The negativity imparted by those Opa proteins predicted to have the most opposite (increased positive charge) affects is due to their adsorbing polyanions (DNA, RNA, heparin, sulfated polysaccharides from agar, and perhaps polyphosphate) from their surrounding milieu. When Opa+ cells attain a 'polyanion capsule,' it greatly reduces their abilities to adhere to epithelial cells in vitro.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00516-05 LMSF

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoglobulin Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S. H. Pincus	Medical Officer	LMSF, NIAID
Others:	C. J. Horgan	Senior Staff Fellow	LMSF, NIAID
	E. I. P. Kamanga-Sollo	Visiting Fellow	LMSF, NIAID
	H. Fang	Visiting Fellow	LMSF, NIAID
	R. L. Cole	Chemist	LMSF, NIAID
	K. Messer	Bio Lab Tech	LMSF, NIAID

COOPERATING UNITS *(if any)*

Z. Pei, Special Volunteer
K. Brown, Special Volunteer, Arthritis Foundation
V. Tolstikov, Contractor

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.25	2.5	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this work is to study the structure and function of the immunoglobulin molecule. The work is being performed so that this understanding will be applied to the development of antibodies for human therapy.

A. Therapeutic systems. 1. Antibodies to surface antigens of group B streptococci (GBS) have been demonstrated to have protective efficacy in a model of neonatal sepsis. We have identified colony opacity variants of GBS and studied their interactions with antibodies and other components of the immune system. 2. The efficacy of anti-HIV antibodies coupled to ricin A-chain has been studied in vitro. Monoclonal and polyclonal antibodies directed against different epitopes have been tested. Biological variants of HIV that escape killing with these immunotoxins and CD4-PE40 have been identified. The phenotype of the cells carrying these HIV has been studied, and the molecular mechanisms of immunotoxin escape have been evaluated. We have also constructed anti-MuLV immunotoxins, and the efficacy of anti-retroviral immunotoxins is being tested in vivo in well-studied animal systems.

B. Genetically engineered antibodies. Vectors have been prepared carrying human constant region genes. These constructs contain both native and altered genes. These constructs have been ligated to variable region genes from antibodies to the synthetic polypeptide (Tyr,Glu)-Ala--Lys. Antibodies were expressed and purified. The antigen-binding capability of these antibodies has been tested. Well-defined immune complexes were constructed using antigens having different epitope densities. The ability of the immune complexes to interact with the complement system has being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00549-04

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Infection with the Lyme Disease Spirochete, *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	P. A. Rosa	Senior Staff Fellow	LMSF, NIAID
Others:	K. L. Tilly	Senior Staff Fellow	LMSF, NIAID
	D. M. Hogan	Microbiologist	LMSF, NIAID

COOPERATING UNITS *(if any)*

N. Margolis, Special Volunteer, Arthritis Foundation
J. Campbell, Stay In School Program
A. Sadziene and A. Barbour, University of Texas at San Antonio

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infection of humans by Borrelia burgdorferi (Bb) can result in a multisystemic, chronic disease with diverse manifestations. We are investigating B. burgdorferi at several levels in order to understand some aspects of the disease pathogenesis. These studies include genetic manipulations of the spirochete and characterization of key structural and regulatory elements.

1. Molecular Characterization: Several features of Bb are being addressed. We have characterized homologous recombination between the genes encoding the major outer surface proteins (Osps) that both deletes osp gene sequences and creates chimeric gene fusions. Bb expressing recombinant osp genes can "escape" killing by Osp-specific neutralizing antibodies. We are also investigating mechanisms of transcriptional regulation of osp gene expression. Isolating and studying genes whose expression is increased after a temperature upshift (heat shock genes) will provide information about their roles in cell growth, pathogenesis, plasmid replication and adaptation to stress. Understanding the mechanism of linear plasmid replication may yield a new target for antibiotics, in addition to shedding light on ways in which organisms manipulate their genetic material.

2. Gene Transfer: In order to allow genetic analysis of Bb, a method for gene transfer into Borrelia is being developed. A derivative of the Bb 16 kb linear plasmid will be used as a vector for electroporation, allowing for maintenance of transferred DNA in a native setting. DNA encoding recombinant Osp proteins that lack neutralizing epitopes will be introduced as a selectable marker.

3. cDNA Library: Extended passage of Bb in vitro eliminates its ability to infect rodents without any obvious differences in protein synthesis. cDNA libraries of high and low passage Bb are being constructed and clones derived from putative low abundance, differentially expressed messages will be identified by subtractive methods. A similar approach will be taken to identify messages that are differentially expressed in response to other biologically relevant stimuli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00568-03 LMSF

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis and Extent of Microbial Sex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: J. A. Heinemann Staff Fellow LMSF, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Beneficial mutations can arise within individuals neutral to the mutation's utility; variants demonstrating the change can then be selectively sorted from the progenitor population by the environment. This observation alone does not exclude the possibility that selective forces can also influence either the generation of genetic alterations, beneficial or otherwise, or the retention of alterations. Inheritable changes can be of at least two types: those that alter the genome by changing its DNA sequence (genetic), and those that establish a self-perpetuating physiological state (epigenetic) in individuals within an otherwise isogeneic population. Both genetic and epigenetic changes that favor a cell's survival may occur in organisms after exposure to an environment.

I have demonstrated the existence of mechanisms that allow eukaryotes to adapt to growth-halting environments after exposure of the organism to the environment. The mechanisms result in the change of genomic DNA sequence at specific (selected) loci nonrandomly. These changes are the result of recombination and require only some of the components of the homologous recombination machinery. Environmentally induced changes in microbes could compromise the efficacy of vaccine-based therapies.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00608-02 LMSF

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression and Phase Variation of Gonococci opa Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R. J. Belland	Visiting Associate	LMSF, NIAID
Others:	T. Chen	Visiting Fellow	LMSF, NIAID
	J. Swanson	Chief	LMSF, NIAID
	S. Morrison	Microbiologist	LMSF, NIAID

COOPERATING UNITS (if any)

W. M. Huang, University of Utah, Salt Lake City, Utah

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	2.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Surface components of the human pathogen Neisseria gonorrhoeae have been shown to change dramatically during the course of gonorrheal disease in male volunteers. The Opa family of outer-membrane proteins change expression states as a result of a unique translational switching mechanism involving the insertion or deletion of discrete numbers of pentameric coding repeat elements (CR) within the segment of the gene coding for the signal-peptide of the Opa pre-protein. The direct, tandem repeat elements have the capacity to form an intramolecular triplex (H-DNA) in response to negative supercoiling torsion. The phase variation frequencies of repeated genetic elements which form H-DNA are significantly lower than elements with the same repetitive nature which are unable to form H-DNA. The enzymes responsible for generation of negative supercoiling (gyrA/B) have been cloned and are being purified to determine their effect on the transcription of opa and the relationship between transcription and H-DNA formation. A number of Opa proteins have been constitutively expressed as beta-lactamase-Opa fusions in E. coli and have been shown to segregate to the outer-membrane in a conformation resembling that seen in N. gonorrhoeae. Recombinant Opa+ bacteria mimic the interactions seen between human neutrophils and gonococcal strains expressing the analogous Opa. Recombinant strains also show an ability to adhere to certain eukaryotic cell lines in in vitro binding assays. One particular Opa fusion protein confers upon the recombinant E. coli strain the ability to adhere to and be taken up by Chang conjunctival epithelial cells. A Type III restriction modification system has been cloned from N. gonorrhoeae MS11. This system may be unique in its expression since at this time it appears that expression of the upstream methylase enzyme is translationally regulated by a series of direct tandem repeats in a manner similar to that found for the Opa system (although this has not been confirmed at this point). If found to operate as a control mechanism for expression this system will provide an interesting contrast to the Opa system with respect to the mechanisms employed for translational regulation by "illegitimate" forms of recombination.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00609-02 LMSF

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthetic Gene Approach to Study the Action of Antibacterial Genes of Mammals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: K. S. Bhat Senior Staff Fellow LMSF, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neutrophil and macrophage cells express several proteins which defend the host from invading bacteria. These proteins are the primary host defense mechanisms. Among them, Defensins are a family of antibiotic proteins whose primary and secondary structures have been elucidated. The primary focus of this project is to understand the Defensin molecules' essential elements that elicit antibacterial activity. A human Defensin gene was chosen as subject. Because artificial genes provide clear advantages for altering genes, the defensin gene was assembled and cloned from synthetic oligonucleotides. During this period, several methods of gene synthesis were evaluated and developed a new method to accurately assemble synthetic genes. The technique involved the synthesis of only one of the DNA strands. The method was optimized by synthesizing the lacZ gene of Escherichia coli. Another aspect of the project involved identification of newer antibacterial genes in mice. For this purpose a cDNA library from mouse macrophage messenger RNA was prepared. This cDNA library is being used to isolate a large number of genes which might produce antibacterial proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00632-01 LMSF

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Siderophores and Iron Transport in *Pseudomona aeruginosa*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R. G. Ankenbauer	IRTA	NIAID, NIH

COOPERATING UNITS *(if any)*

C. White, Stay in School Program

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The transport of Fe(III)-pyochelin by Pseudomonas aeruginosa has been investigated. The receptor for Fe(III)-pyochelin has been determined to be a ~75 kDa outer membrane protein. Mutants lacking this outer membrane protein have an absolute defect in the transport of Fe(III)-pyochelin. This finding refutes the previous designation of a 14 kDa outer membrane protein as the receptor for Fe(III)-pyochelin. Work on pyochelin analogues has resulted in the discovery of a novel artificial siderophore and iron transport system in P. aeruginosa. Although this artificial siderophore, trivially designated HTC, has structural similarities to pyochelin, its transport is independent of the Fe(III)-pyochelin receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00633-01 LMSF

PERIOD COVERED

October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neisserial-Host Interaction: Pathogenic Mechanisms and Host Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S. H. Fischer Research Associate LMSF, NIAID

COOPERATING UNITS *(if any)*

M. Konkel, LVP, RML, NIAID

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neisseria gonorrhoeae interactions with human polymorphonuclear neutrophils and host proteins were studied in order to understand how the expression of different gonococcal surface components affects bacterial association with host cells and cell surface proteins. Studies were carried out to characterize A) gonococcal interactions with human extracellular matrix proteins and B) the role of fourth oligopeptide loop of gonococcal opacity proteins in mediating neutrophil (PMN) responses to gonococci.

A. Attachment of gonococci to extracellular matrix proteins: Gonococcal with or without opacity (Opa) proteins were tested for adherence to plastic coverslips coated with human laminin, vitronectin, fibronectin (FN), collagen type IV or bovine serum albumin (BSA). Neither Opa+ nor Opa- variants showed increased adherence BSA compared to the plastic coverslips alone. Opa+ variants, but not Opa-, adhered significantly more to FN than to BSA. Gonococci expressing Opa A showed 1.8X, 3.8X and 11.2X times greater adherence to laminin, vitronectin and FN, respectively, than to BSA but no increased adherence to collagen type IV. Rabbit anti-human FN antiserum inhibited Opa A attachment to FN by 50% while normal rabbit serum had no effect. Interestingly, PMN responses to Opa+ gonococci were inhibited in a dose-dependent manner by added FN.

B. Role of the gonococcal opacity protein fourth loop in mediating human neutrophil responses to gonococci: A peptide representing the conserved surface-exposed Opa protein fourth loop inhibited neutrophil responses to gonococci in suspension and when coated on surfaces directly stimulated neutrophil responses; a control peptide with a scrambled sequence did neither. Rabbit antisera against the fourth loop peptide was opsonic for Opa+ gonococci and affinity-purified anti-fourth loop IgG inhibited PMN non-opsonic responses to gonococci. This suggests a role for the fourth oligopeptide loop in neutrophil interactions.

LABORATORY OF PARASITIC DISEASES
1992 Annual Report
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Laboratory of Parasitic Diseases
National Institute of Allergy and Infections Diseases
Summary -- October 1, 1991 - September 30, 1992

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

The big administrative and organizational story in the past year was the creation of a separate Laboratory, the Laboratory of Malaria Research (LMR) from the previous Malaria Section of LPD. Dr. Louis Miller is Chief of LMR and the staff of the former Malaria Section of LPD simply have been transferred over to LMR. Functionally, the short-term consequences of the division of LPD are not very large since the Malaria Section has operated quite independently for many years, and only a few physical dislocations are planned. LPD and LMR will continue to share certain facilities, such as the large common equipment room and certain secretarial services. It is anticipated that the close research interaction and collaboration of staff of the two labs that has existed in the past will continue. Participation in the out-patient clinical activities and clinical teaching conferences of LPD will be open to interested staff of LMR. The LPD weekly research seminars will be continued under joint LPD-LMR sponsorship. Therefore, we hope that individuals who come to either LPD or LMR for research training will be exposed to information and research developments concerning all of the parasitic diseases under study in both laboratories.

With the creation of LMR, and consequent reduction in size of LPD, it became possible for Dr. Neva to take on a new administrative responsibility as Director of the Intramural Center for Tropical Diseases. He will represent the NIAID intramural laboratories, principally LPD, LMR and the Laboratory of Infectious Diseases (LID), in a new linkage of both Intra- and Extramural programs in the areas of parasitology and tropical diseases.

The Extramural programs include 7 institutions with the International Collaborations in Infectious Disease Research (ICIDR) grants, 4 groups that have Tropical Disease Research Unit (TDRU) grants, and 3 new Tropical Medicine Research Centers (TMRU), for a total 14 groups supported by NIAID with international research programs. In addition, there are 9 academic institutions with research interests in parasitic diseases, but without specified foreign collaborators. The Directors of these groups will meet annually for the purpose of advising the Institute on progress in tropical disease research, discussion of new approaches for international interactions, and promotion of public interest in the field. The first such meeting was held in late April-early May.

Departing professional personnel included two IPA guest researchers, Donald Krogstad, M.D. from Washington University in St. Louis who worked mainly with the Malaria program, and Steve Williams, Ph.D., from Smith College in Massachusetts, who worked in the Clinical Parasitology Section. Dr. Krogstad will assume a new job as Chairman of the Department of Tropical Medicine at Tulane University, and Dr. Williams returns to his home institution. Chris Karp, M.D., an NRSA Fellow left to take an Infectious Disease Fellowship at Johns Hopkins University, and Andrea Cooper, Ph.D. has accepted an academic position at Colorado State University. Dr. Zilton Andrade, the Fogarty Scholar from Brazil finished his second visit with LPD. Vicky Mann, Ph.D. resigned her IRTA Fellowship so she could join her husband-to-be, Paul Brindley (a recent LPD Fellow) in

Australia--which shows that LPD serves functions other than biomedical research! Dr. Nozaki will leave for another Fellowship at Rockefeller University before returning to Japan.

It is little wonder that LPD labs are crowded since our arrivals over the past year exceed departures by almost two to one. Dennis Dwyer's lab received 3 new Fellows, Alain Debrabant, Ph.D. from Belgium, Greg Russell, Ph.D. from Australia, and Mary Alleman, Ph.D. from the U.S.A. Newcomers to Alan Sher's lab were Tom Wynn, Ph.D. and Lena Aslund, Ph.D. from Sweden. David Sacks' lab received Lucia Carrera, a Ph.D. veterinarian from Spain and a return engagement from Elvira Saraiva, Ph.D. from Brazil. Another arrival who will work both with Dr. Sacks and Dr. Neva is Richard Kenney, M.D., who did molecular biology in Dr. Gallins' lab for several years and now wishes to work on leishmaniasis. Two new arrivals to work with Dr. Nash are Hugo Lujan, Ph.D. from Argentina and immunologist Linda Byrd, Ph.D. Peter Zimmerman, Ph.D. comes to join the Clinical Parasitology Section as an NRC Fellow, and Y. Miyahira, Ph.D. from Japan came to work with Dr. Dvorak.

The international make-up of LPD is evident from the fact that people from 10 different countries are represented (Sweden, Belgium, England, Australia, Argentina, Brazil, Spain, Japan, France and India).

There were 4 research projects carried out abroad by LPD personnel during the past year. Drs. Ottesen and Mahanty worked on filariasis in India, Drs. Nutman and Steel initiated work on onchocerciasis in Ecuador, Drs. Sacks and Karp carried out studies on visceral leishmaniasis in the African Sudan, and a virtual expedition was mounted on the island of Mauke of the Cook Island group by Dr. Ottesen with the help of Drs. McCarthy and Steel and Mr. Poindexter. The Cook Island study is a unique long-term follow-up of a project carried out 17 years earlier. Needless to say, these studies were possible only with the helpful collaboration of foreign colleagues.

HONORS AND AWARDS

All of the permanent professional staff, and many of the senior non-tenured staff serve on Editorial Boards of scientific journals and/or frequently review manuscripts for journals. Such activities reflect the high standing of LPD scientists by professional peers. Also many of the staff serve on Ad Hoc Committees for a granting agencies or foundations, and are invited to participate in workshops and scientific meetings. Such activities are generally not cited here individually unless felt to deserve special mention.

Dr. Sher was perhaps the most active of LPD staff on the national and international lecture circuit, giving invited talks or name lectures at the N.Y. Blood Center, at Dartmouth and the University of Texas at San Antonio, and the B. McGhee Lecture for the Am. Soc. of Parasitologists. He was one of the organizers of the First African Congress of Immunology held in Nairobi, and serves on the Burroughs-Wellcome Fellowship Committee. Drs. Cheever and Sher were invited speakers at the Int'l Congress of Schisto in Brazil. Dr. Nutman was a Wellcome Visiting Scientist at Imperial College in England, and also served on an Ad Hoc committee for WHO/TDR. Dr. Neva spoke to FDA and the Blood Banking Assoc. on dangers of transmission of parasites by transfusion.

RESEARCH PROGRESS

Immunology and Cell Biology Section

Tangled cytokine trail in experimental Schistosomiasis. The down-regulation produced by IL-10 involves interaction with other cytokines. Last year it was concluded that the cytokine IL-10 was responsible for suppressing INF-gamma activation of macrophages in killing schistosomulae. The actual mechanism was shown to be inhibition of production of toxic nitric oxide (NO) metabolites. But IL-10 was also found to block production of another cytokine, TNF-alpha. In addition, the cytokines TNF-beta and IL-4 were found to act with IL-10 in inhibiting activated macrophages via a pathway independent of TNF-alpha (Oswald, James and Wynn). So, the mechanism of IL-10 immunosuppression is more complex than first surmised.

New method of semi-quantitative PCR to assay transcription of cytokines. By extraction of MRNA from tissue specimens, reverse-transcribing it, and performing PCR using primers specific for various cytokines, it is possible to detect presence and estimate relative amounts of cytokines expressed in the specimen. This technique was applied to schistosome egg granulomas in mouse lungs. Results with this approach, plus the use of anti-cytokine monoclonal antibodies, suggest that IL-4 and other Th2 cytokines may be the most critical for granuloma formation (Wynn and Eltoum). This technique should be especially useful in following immunologic events over time and under conditions in which cytokines are either consumed, broken down, or otherwise difficult to assay directly.

Th1 suppression produced by schistosome infection results in delayed clearance of concomitant virus infection. Schistosome infected mice later infected with a recombinant vaccinia virus were found to have greatly delayed clearance of the virus from the liver, spleen and lungs. Since the animals showed inhibition of cytolytic T cell responses to vaccinia, results were attributed to a suppressed Th1 response that occurs in the early stages of the schistosome infection. Whether schistosomiasis in humans has the same effect on certain viral infections, such as hepatitis B, can only be speculated (Actor, Buller of LVD, NIAID and J. Berzofsky of MEI, NCI).

Search for Schistosome vaccine continues. New candidate recombinant antigens. The recombinant expressing paramyosin in a BCG vector was able to induce a Th1 type cell-mediated response, but gave no significant protection in vaccinated mice. Negative results may have been due to the low level of paramyosin expressed by the vector and a slower growth rate by the BCG construct than by wild type BCG. New recombinants have been constructed and will soon be tested; one expressing cercarial elastase and another expressing glutathione-S-transferase (Actor, Oswald, Molecular Vaccines, Inc. and J. McKerrow of Univ. of Calif).

Use of T cell lines and clones to identify protective antigens. T cell lines and clones were produced from mice that had been immunized with soluble whole worm antigen plus BCG. One T cell clone (B) and one T cell line (1S2) were derived which in cell transfer experiments confer protection. Clone B gives a Th1 patter of cytokines and the 1S2 line shows predominantly a Th2 cytokine pattern. Neither the cell line or clone recognize paramyosin. Expression clones from a CDNA library are being screened for cytokine responses from the T cell clone to try to identify the relevant antigen(s) (Oswald, James and Williams).

Immune regulation in experimental toxoplasmosis. Identification of T. gondii antigen responsible for T cell cytotoxicity and pathway of Class I MHC presentation. Several approaches are being used to identify the parasite antigen recognized by Cd8+ effector cells that result in cytotoxicity of toxoplasma infected cells. One approach is to fractionate soluble T. gondii antigen which reacts with infected bone marrow macrophages and destroys them. The other approach is to identify peptides that sensitize L cells transfected with Class I MHC for recognition of cytotoxic lymphocytes. These results suggest a requirement for cellular internalization in the processing of soluble antigen by bone marrow macrophages for Class I restricted recognition (Denkers, Gazzinelli).

Down regulation of macrophage mediated killing of T. gondii and T. cruzi by cytokines. Both IL-10 and IL-4 were found to inhibit killing of intracellular toxoplasma by INF-gamma activated macrophages in a dose-dependent fashion. The actual mechanism of parasite destruction was by nitrogen oxides (NO). TGF-beta had even more potent down-regulatory effects, and its action was potentiated by IL-10 and IL-4. (Gazzinelli and Morse of LIP, NIAID). The same cytokines (IL-4, IL-10 and TGF-beta) were shown to inhibit killing of T. cruzi by INF-gamma activated macrophages (Gazzinelli and Oswald).

Role of NK cells in cytokine response to Toxoplasma. The main cytokine mediator of resistance to Toxoplasma is considered to be INF-gamma. In order to examine the role played by NK cells in the INF-gamma response, spleen cells from B and T lymphocyte.-deficient mice were found to produce INF-gamma on exposure to Toxoplasma tachyzoites. The INF-gamma response required viable organisms, and was abolished by depletion of NK cells, and required macrophage accessory cells. SCID mice depleted of NK cells are also less resistant to toxo than untreated mice. Thus, NK cells clearly can be activated to produce INF-gamma (Gazzinelli, Sher and Oswald).

Cell mediated responses in Leishmanial infection. Cytokine mRNA in bone marrow of patients with visceral leishmaniasis. It is well-known that parasite antigen-specific anergy, as measured by delayed hypersensitivity skin tests and lymphocyte proliferation, is a feature of active visceral leishmaniasis. T cell responsiveness returns a variable period of time (months) after successful treatment. For further insight into possible mechanisms of this anergy and the role of cytokine regulation in affected tissues, cytokine mRNAs were analyzed from the bone marrow of patients. The procedure involved extraction and reverse transcription of RNA, followed by semi-quantitative PCR using primers specific for various cytokines. PCR products were analyzed by gel electrophoresis, followed by Southern transfer, probing with a non-radioactive detection system and densitometry to quantify the signal. Relatively high levels of mRNA for IL-10 were found in the bone marrow of active visceral patients, with a fall to negative or barely detectable levels after treatment, as were patients with other diseases or normal volunteers. mRNA for INF-gamma could also be detected, but since IL-10 in other systems can inhibit macrophage activation, we believe IL-10 may play an important role in the immunosuppression of active visceral leishmaniasis (Karp, Sacks, various Sudanese collaborators, and Michigan State and Brigham Young Univ. ICIDR program collaborators).

Analysis of T cell responses to leishmanial antigens in-vitro. Frozen stored peripheral blood mononuclear cells from patients with cutaneous and mucocutaneous leishmaniasis were thawed and used to assay reactivity to different antigen preparations by proliferation and by production of INF-gamma. Amastigote infected monocytes, as well as soluble leishmanial antigen (L. donovani), showed positive responses. T cell depletion showed that both responses were due primarily to CD4 cells. Some evidence for a greater INF-gamma response to "live" amastigote antigen was seen with the cells of mucocutaneous patients (Cooper, Sacks, Melby, Karp and Neva).

Cell-mediated immunologic (CMI) responses in "Desert Storm" veterans with atypical systemic leishmaniasis. Walter Reed Army Medical Center (WRAMC) and Navy physicians have referred patients with proven (positive bone marrow culture) and suspected visceral leishmaniasis to us for evaluation of CMI in efforts to establish diagnostic criteria.
Five of the seven bone marrow isolates and one of two lymph node isolates that could be typed as to species were found to be L. tropica, a species that normally causes cutaneous disease. Many of the clinically suspect cases show a variable pattern of significant lymphocyte proliferative responses to leishmanial antigens. Only a few of the cases exhibit positive delayed hypersensitivity skin test (leishmanin) responses. The aggregate experience strongly suggests that considerable numbers of military personnel have been infected with leishmanial parasites during the 3-6 months exposure in the Persian Gulf, and have experienced an atypical, difficult to characterize, disease (Neva, Magill and Oster at WRAMC and Ohl at Navy).

Cell biology of leishmanial development. Molecular basis for leishmanial vector specificity. Last year the postulated changes that occur in the lipophosphoglycan (LPG) surface molecule of L.major as it grows in the sandfly gut were described. These changes were based upon lectin binding profiles and/or structural analysis of LPG purified from organisms grown in vitro. The LPG developmental polymorphisms have now been studied in additional leishmanial species, L. donovani, L. tropica, L. infantum, and L.b. amazonensis. In each case the molecule undergoes a 2-3 fold increase in the number of phosphorylated oligosaccharide repeat units expressed, and in each case terminally exposed sugars are modified. Three categories of structural modifications have so far been defined: (1) substitution and/or capping of terminal side chain sugars (L. major), (2) loss of terminal side chain sugars (L. amazonensis) and (3) substitution and/or loss of neutral capping sugars (L. donovani) The parasite species specificity of different sandfly vectors was demonstrated by experiments in which parasite development associated with LPG modification determined loss of parasite binding to sandfly midguts. This permits the infective parasite to migrate anteriorly to mouth parts. Also, LPG from L. major was shown to bind to P. papatasi midguts but failed to bind to P. argentipes midguts, whereas L. donovani did just the reverse. P. papatasi is the vector for L. major and P. argentipes is the vector for L. donovani. (Sacks, Pimenta, Turco of Univ. Kentucky and McConville of Univ. of Dundee, and Rowton of WRAIR).

Conditions affecting infectivity of leishmania in-vitro and in-vivo. Increasing the surface: volume ratios of parasite growth in-vitro (i.e., growth in minimal volumes) was found to promote metacyclogenesis (i.e., transformation to infective forms), of leishmanial promastigotes. The effect of salivary gland products on infectivity for mice is also under investigation (Sacks, Carrera, Saraiva and Cooper).

Characteristics of in-vitro grown Leishmanial amastigotes. Evidence for similarities between in-vitro grown "amastigotes" and promastigotes. As reported last year, a strain of L. donovani was adapted to growth at high temperature (37^{0}C) and resembled morphologically the intracellular form, or amastigote (Am-), instead of flagellate form or promastigote (Pro-) that normally grows in culture at 26^{0}C. Several additional L. donovani isolates, as well as 4 more species of Leishmania (L. tropica, L. major, L.m. mexicana, and L. infantum) have now been adapted to grow as amastigotes. (Dwyer). By light microscopy, scanning and transmission EM the in-vitro Am- were morphologically identical to in-vivo derived amastigotes (Dwyer and Pimenta). The doubling times of both cell phenotypes had virtually identical mean generation times of about 11.2 hours, and during log phase growth each phenotype had approximately 60%, 15% and 25% of its population in G_1, S and G_2-M phases of cell cycle, respectively (Dwyer and Dvorak).

Evidence for differences between in-vitro "amastigotes" and promastigotes. Although Pro- and Am- forms were found to have many common surface membrane proteins, some were unique, as demonstrated by ^{125}I-surface labeling and immuno-precipitation. An isoform of 66 kDa membrane protease was seen in Am-, as well as the usual GP-63 seen in Pros-. Also, Am- produced an isoform of secretory acid phosphatuse similar to that produced by in-vivo amastigotes. Most of the labeled Am- surface membrane components were immunoprecipitated by sera from Kala-azar patients (Dwyer).

Comparative activity of enzymes expressed by Pro- and Am- L. donovani. Specific activity of 3$'$ nucleotidase of Am- was 2-fold lower than in Pro-. Am- showed a unique band of protease activity at 100 kD and the total protease activity of Am- was 5-fold higher than in Pro- (Dwyer, Alleman and Debrabant).

Protein methylation in Trypanosomatids. Methylation of surface membrane proteins in response to adenosine starvation. Both Leishmania and Crithidia parasites were found to rapidly modify surface membrane proteins by methylation in response to stress conditions, such as adenosine starvation. The protein methylating agent (i.e., methyl group donor) for both parasites was found to be S-adenosylmethionine (SAM). The kinetics of this process was characterized using tritiated (^{3}H)-methyl SAM. Results demonstrated that a 10-fold difference in the rate and extent of protein methylation between replete and adenosine starved cells. Cells starved for adenosine for as little as 15 minutes showed a significant elevation in protein methylation index. These observations provide the first evidence for an environmentally triggered regulatory mechanism of these parasites (Alleman, Bacchi of Haskins Lab of Pace Univ., Gottlieb and Dwyer).

Host-Parasite Relations Section

Experimental pathology of schistosomiasis. Further studies of influence of cytokines on hepatic fibrosis and schistosome egg granulomas. Last year it was shown that inhibition of IL-2 by monoclonal antibody (Mab) resulted in a dissociation of Th2 type lymphocyte function in schistosome infected mice (S. mansoni). In other words, serum IgE levels were increased, but blood and tissue eosinophilia were decreased. The effect of anti IL-4 treatment was studied next and found to markedly decrease hepatic fibrosis in either S. mansoni or S. japonicum infections of mice. The size of S. mansoni egg granulomas was modestly decreased, but was somewhat increased for granulomas of S. japonicum. Thus, Th2 type responses appear to be important in the genesis and regulation of schistosomal hepatic fibrosis (Cheever, Sher and Finkelman of USUHS). The influence of other cytokines in this model system are planned.

Fecundity of S. mansoni and S. japonicum in mice. Since the pathologic effects of schistosome infection on the host are generally proportional to the number of eggs laid, it is important to have reliable estimates of just how many eggs are produced by each species of parasite. Single pair infections of S. mansoni in mice showed an average of 330 eggs per day per female worm over a period of one year. Fecundity decreased somewhat with time in more heavily infected animals. With S. japonicum infected mice egg production decreased from over 2000 per day per worm to less than 1000 per day in animals infected with 1 or more pairs. Eggs trapped in tissues, as well as eggs passed in feces were counted and fecal excretion with both species was found to be a reasonable reflection of total eggs produced (Cheever and Mosimann of DCRT).

Molecular classification of Giardia isolates. Different animals have their own species of Giardia parasite, but sufficient differences have been found among the Giardia isolated from humans to classify them in 3 groups. 18S ribosomal DNA sequences were identical within a group but different between groups. Sequence differences in the triosephosphate isomerase genes of two isolates differed by 19 per cent; a genetic divergence equivalent to that between man and mouse! One gene transcript was found to be present in only one of the 3 groups of Giardia. The genomic organization of VSP genes can also differ among isolates (Nash, Mowatt)

Immunology and biochemistry of Giardia. A metal binding domain, presumably for Zn^{++} has been found in sequences of most of the variable surface proteins of Giardia. This raises the interesting question that the parasite may inhibit Zn^{++} absorption, or inhibit critical intestinal enzymes. Since the variable surface proteins are rich in cysteine, the studies of the metabolism of this amino acid have been initiated. The prospects of developing a convenient animal model for Giardia infection that resembles that of the human have improved. A particular strain of parasite was found that produces an initial heavy infection in adult mice and then persists at a much lower level for several months. If this finding is confirmed it opens up a wide range of studies, including examination of immunologic and genetic factors that influence infection (Byrd, Nash and Mowatt).

Clinical Parasitology Section

Use of molecular biology to identify diagnostic antigens. Since filarial parasites are antigenically complex, and crude antigen preparations will frequently give immunologic cross-reactions, preparation and use of cDNA libraries to identify more specific antigens is a logical approach. This was very successful with the Onchocerca volvulus (OV16) recombinant antigen reported last year, which is now undergoing extensive field tests by WHO (Lobos, Ottesen, Nutman). A similar approach has been taken with a cDNA expression library from adult female W. bancrofti worms to screen for diagnostic antigens. More than 25 potential clones have been identified and are being characterized further (Raghavan and Nutman). Microfilarial antigens of Loa loa parasites are being screened by 2D immunoblotting with human and experimentally infected animal sera. An excretory/secretory antigen from Loa microfilariae appears to be a promising candidate against which anti-serum can be prepared for screening an existing expression library (Elson and Nutman)

Search for protective or "pathogenic" antigens with molecular probes. In geographic areas heavily endemic for various parasitic infections, some individuals are frequently identified who have had considerable exposure to a parasite and yet appear to be free of infection, or immune. This situation has been encountered in Guatemala as well as in more recent studies on onchocerciasis in Ecuador. Such putatively immune cases generally show a very strong cell-mediated as well as humoral immune response. Therefore genomic expression libraries were screened with pools of sera from "immune" and non-immune individuals. Several clones have been identified which reacted with all sera from "immunes" and only 2 of 25 sera from infected patients. Characterization of the native antigens for which the molecular clones code is underway and could provide the basis for a future vaccine (Nutman and McCarthy).

A reverse situation may apply in the case of several antigens representing microfilarial proteins which react with serum pools from patients with tropical pulmonary eosinophilia. By Western blots these antigens were shown to be recognized by IgE and IgG4 antibodies. These antigens also are capable of inducing IgE antibodies in-vitro and IL-4 in T cells of sensitized patients. In this case the antigens identified may be implicated in the pathogenesis of the tropical pulmonary eosinophilia syndrome (Lobos, Ottesen and Nutman)

Immunoregulation in filarial infections. The immunoregulatory events in response to parasite antigens was reported last year. An IL-4 T cell response leads to an expansion of B cells producing all classes of IgG as well as IgE. Later, there is a switch to production of IgG4. The molecular mechanism for this isotype switching is being investigated using PCR with primers capable of demonstrating epsilon heavy chain switch events (Mollis and Nutman).

Attempts are being made to identify the CD4 cell population involved in production of Th2 type cytokines by 3 color flow cytometry. On CD45RO+ cells (Memory T cells) a HLA-DR+ cell population was identified which showed presence of known activation markers. Evidence for Th2 type cytokines in these putative Th2 cells will be sought using reverse transcriptase PCR. (Elson, Nutman, and Shaw of NCI). The onchocerciasis studies in Ecuador provide an opportunity to examine whether immunity to this infection is HLA restricted. Infection rates are much lower in one racial group compared to another living under conditions of apparently equal exposure (Elson, Zimmerman and Nutman with Guderian in Ecuador).

Diagnosis and treatment of strongyloidiasis. Efforts to purify the larval protease, which could have great potential as a diagnostic antigen, were unsuccessful, so a new approach was taken. A cDNA library was prepared and several clones reacting with a serum pool from patients have been isolated. Both react with IgG and IgE antibody. (Raghavan, Gam and Neva). Several additional HTLV-1 infected patients who also have strongyloidiasis have come to our attention. They are being investigated for evidence of some type of selective immunodeficiency that would help explain failure to be cured of the strongyloidiasis by treatment. (Neva and McFarlin of NINDS). The comparative trial of strongyloides treatment in Lowell, Mass. with thiabendazole vs. ivermectin in Cambodian refugees, in which we are collaborating, officially ended in July. Follow-up serologic tests have not yet been completed and the tabulation of results is not yet available to us (P. Gann of Univ. of Mass. and Neva).

Physiology and Biochemistry Section

Characterization of a triosephosphate isomerase in Giardia. A key enzyme of carbohydrate metabolism, triosephosphate isomerase (TIM) was found to be present in Giardia representing two different groups. Genes for TIM were cloned and transfected into a mutant strain of E. coli lacking the enzyme. The cloned genes functioned normally in the transfected E. coli (Weinbach, Mowatt and Levenbook). Attempts to purify TIM from Giardia, which differs markedly in amino acid sequence from other organisms, are continuing (Weinbach and Levenbook).

Variation in DNA content of hemoflagellate parasites. Studies of the DNA variation occurring in a single cell clone-derived strain of T. cruzi that was reported several years have been expanded. The presence of non-nuclear, non-kinetoplast DNA can be demonstrated in the cytoplasm of cells by low-light-level video microscopy in other strains of T. cruzi. Environmental changes, especially in temperature, can induce the changes in DNA, which have been demonstrated in Leishmania and Crithidia species as well. The changes are not related to heat-shock protein. The biological significance of the DNA changes is not known (Nozaki, Dvorak and Mudd of BEIP).

Invasion of red cells by malaria parasite revisited. Low-light-level video microscopy which helped provide clues to existence of receptors for the parasite nearly 20 years ago is being used again to clarify the origin of the parasitophorous vacuolar membrane during invasion. Quantitative estimates of the concentration of fluorescent lipophilic probes in the parasitophorous and erythrocyte membranes are identical. Other probes to label specific components of the parasite are being investigated (Dvorak with Ward and Plowe of LMR and Mudd and Casio of BEIP).

Parasite Growth and Differentiation Section

More on the evidence that non-pathogenic E. histolytica is a separate species. In addition to the differences in small subunit ribosomal RNA patterns and in isoenzyme patterns between pathogenic (P) and non-pathogenic (NP) E. histolytica that has become apparent in the last few years, inability to convert NP strains to axenic growth has been another differential feature. In the case of P isolates they can be routinely axenized, while numerous attempts to axenize NP isolates by the same method have failed. Recently, an NP strain of parasite has been grown in the presence of glutaraldehyde fixed E. coli or C. fasciculata. If this can be done with a few additional NP strains it may then be possible to look for metabolic or other biochemical differences between P and NP forms. The cumulative evidence now for differences in P and NP forms justifies redesignation of the species as E. histolytica (Schaudinn, 1903) for the pathogenic variety, and E. dispar (Brumpt, 1925) for the non-pathogenic form (Diamond and Clark).

Does E. histolytica have a mitochondrion after all? Because E. histolytica lacks cytochromes and a functional TCA cycle, and has no morphologic evidence of such an organelle, it has been considered to lack mitochondria. Years ago an enzyme with the activity of a pyridine nucleotide transhydrogenase was detected in the particulate fraction of amebae. This enzyme in some organisms is associated with mitochondrial membranes. With recently published sequence data from bacterial and bovine mitochondrial transhydrogenase genes, a related gene was sought in E. histolytica by PCR and was found! Whether this constitutes sufficient evidence to re-open the mitochondrial question in amebae is being studied (Clark, Diamond and Weinbach).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00094-33 LPD

PERIOD COVERED
October 1, 1991 to September 30,.1992

TITLE OF PROJECT
Entamoeba histolytica: Cellular physiology and molecular diagnostics.

PRINCIPAL INVESTIGATOR	L. S. Diamond	Section Head	LPD, NIAID
Others:	C. G. Clark	Staff Fellow	LPD, NIAID
	D. Mirelman	Fogarty Scholar	FIC
	E. C. Weinbach	Section Head	LPD, NIAID

COOPERATING UNITS *(if any)*
National Zoological Park (Ann Bratthauer)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Parasite Growth and Differentiation

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.3	PROFESSIONAL: 2.3	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The dual nature of *Entamoeba histolytica*, as currently defined, has been further examined. One of the implications of the proposed conversion of NP amebae to the P form is that stresses encountered during the axenization process trigger the conversion. Several attempts to axenize NP isolates by methods which routinely produce axenic cultures of P isolates all failed. Axenization of a single NP isolate was finally achieved using a novel axenic culture system. Most important, this NP isolate after several months of axenic cultivation has not converted to a P as determined by isoenzyme and riboprinting analyses. Moreover, it differs physiologically from axenized P isolates. This difference, along with the many genetic differences, supports the concept that the two forms are distinct species, *E. histolytica* Schaudinn 1903 and *E. dispar* Brumpt 1925.

That *E. histolytica* lacks mitochondria is accepted dogma. However, recently published phylogenetic trees imply that *Entamoeba* is descended from mitochondria-bearing ancestors. A search in *E. histolytica* has revealed the presence of a gene that in other eukaryotes encodes a mitochondrial protein. This finding has important implications for our understanding of the metabolism of the parasite.

Electrophoretic isoenzyme analysis plays an increasing role in distinguishing between the several species of amebae infecting the human gut. Limitations associated with the use of starch gels, which is the present standard, have been overcome through use of agarose gels. Proteose Peptone #3 can substitute for casein peptones, which are routinely used in media for axenic culture of *Entamoeba*.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00097-34 LPD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT

Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR	T. I. Mercado	Rsrch. Physiologist	LPD, NIAID
Others:	H. M. Fales	Chief, Lab of Chem	IR, CH, NHLBI
	V. J. Ferrans	Chief, Ultrastructure Section	IR, PA, NHLBI

COOPERATING UNITS *(if any)*

Millipore-Waters Life Science Laboratory, Fairfax, Va. (J. Stone); Department of Chemistry, Georgetown University, Washington, D.C. (G. Hammer); MacArthur Center for Molecular Parasitology, Yale University School of Medicine, New Haven, CT. (S. Aksoy).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Studies on the purification and chemical characterization of a lytic, anti-trypanosomal factor (ATF-II) from the bacterial species, *Pseudomonas fluorescens*, were continued. Elemental microanalysis, reversed-phase high performance liquid chromatography (HPLC), Sephadex gel filtration, gel electrophoresis, fast atom bombardment mass spectroscopy (FAB), and nuclear magnetic resonance spectroscopy (NMR) were employed. The purified ATF-II consists of three main components, an alpha-d-glycoside, a peptide, and a component that was not identified, but appears to be a contaminant. More precise HPLC is being used to eliminate this fraction and facilitate an exact determination of the chemical structure. The determination of the chemical structure is essential before studies on synthesis are initiated and pharmacological and toxicological properties are examined employing *Trypanosoma cruzi* infections.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-884-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00098-36 LPD

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.C. Weinbach	Section Head	LPD, NIAID
Others:	L. Levenbook	Research Chemist	LPB, NIDDK
	L. Diamond	Research Zoologist	LPD, NIAID
	M. Mowatt	Senior Staff Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the biochemical and genetic characteristics of a key enzyme of carbohydrate metabolism, triosephosphate isomerase (TIM), in trophozoites of *Giardia lamblia*, were continued this year. In collaboration with Dr. Levenbook, purification of the enzyme was undertaken. Ammonium sulfate fractionations followed by chromatographic separation of the enzyme from other soluble proteins resulted in a 100-fold enrichment of TIM activity. Chromatography revealed that the parasite enzyme has properties which differ markedly from mammalian and trypanosome counterparts.

In collaboration with Dr. Mowatt, transfection experiments with the TIM gene from *G. lamblia* and a TIM chromosomal deletion mutant of *Escherichia coli* showed that TIM activity was now expressed by the mutant bacterium. Complementation with TIM genes suggests the general applicability of the method to cloning other genes for which *E. coli* mutants are available.

Collaborative studies with Dr. Diamond on the respiratory activities of *Entamoeba spp.* were completed this year. Only the low temperature reptilian species of amoeba exhibit specific and marked stimulation of NAD(P)H oxidase by Mn^{2+}. Collaboration with Dr. Clark, who has cloned and partially sequenced the gene for transhydrogenase in *E. histolytica*, showed that this enzyme is associated with the particulate fraction of the parasite. Since this enzyme previously has been found only in cells containing mitochondria, these observations may indicate a pre-mitochondrial component of a parasite lacking this organelle.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00099-22 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Biophysical Parasitology

PRINCIPAL INVESTIGATOR	J. A. Dvorak	Res. Microbiologist	LPD, NIAID
Others:	J. P. McDaniel	Fogarty Fellow	LPD, NIAID
	T. Nozaki	Fogarty Fellow	LPD, NIAID
	Y. Miyahira	Fogarty Fellow	LPD, NIAID
	D. W. Alling		OSD, NIAID
	C. V. Mudd	Senior Engineer	ACE, BEIP
	H. Casio	Engineer	ACE, BEIP

COOPERATING UNITS *(if any)*
Biomedical Engineering and Instrumentation Branch, DRS;
Office of the Scientific Director, NIAID; Biological Resources Branch, Division of Intramural Research, NIAID; Laboratory of Malaria Research, NIAID

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

This project is concerned with (a) analyses of the genetic diversity of medically important parasitic protozoa and its implications to the epidemiology, course and diagnosis of disease, (b) the development of high resolution flow cytometry and quantitative low-light-level video microscopy instrumentation for biomedical research and (c) the utilization of flow cytometry and low-light-level video microscopy for the analyses of infectious agents. We have found that DNA variants of *Trypanosoma cruzi* appear within a cloned stock at low frequency. The DNA of these variants is unstable. Variant populations contain members with high proportions of non-nuclear, non-kinetoplast DNA in the form of particles in the cytoplasm. Environmental variables such as temperature can act to destabilize the DNA of *T. cruzi* resulting in the appearance of variant stocks. These observations imply that cytokinesis without karyokinesis followed by DNA stabilization is occurring in this species. The ability of *T. cruzi* to form viable hybrids in this manner could promote intra-specific heterogeneity and provide a basis for the diversity observed in the presentation and course of Chagas' disease. Low-light-level video microscopy studies are elucidating the chemical composition of the parasitophorous vacuole formed during the infection of erythrocytes by malaria merozoites.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER
Z01 AI 00102-18 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR	F. A. Neva	Chief	LPD, NIAID
Others:	D. Sacks	Rsrch Microbiol.	LPD, NIAID
	C. Karp	NRSA Fellow	LPD, NIAID
	A. Cooper	Visiting Fellow	LPD, NIAID
	R. Kenney	Sr Rsrch Investigator	LPD, NIAID

COOPERATING UNITS *(if any)*
Med. Rsrch Council of Sudan (M. Hag-Ali); Rajendra Mem. Rsrch. Inst., Patna, India (Prabakhar); Min. of Health, Honduras (C. Ponce); Walter Reed Army Med. Center (C. Oster & A. Magill); Dept. of Biology, Youngstown State U (R. Kreutzer) & Yale U School Public Health, Dept. Epidemiology, New Haven, CT (D. McMahon-Pratt); Ntl Naval Med Center, Bethesda (C. Ohl)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

This project continues to focus upon the different clinical forms of leishmanial infections in humans, the cell-mediated immune responses to leishmanial antigens, and characteristics of the causative parasites.

Six parasitologically proven cases of cutaneous leishmaniasis were diagnosed and treated at the NIH Clinical Center during the past year. Many additional patients referred to the NIH by local military services (Walter Reed Army Medical Center and National Medical Center) were evaluated by cell-mediated immunologic studies and leishmanin skin tests for evidence of past or present leishmanial infection. Many of these cases were leukophoresed to obtain lymphocytes for research purposes. *Leishmania tropica*, a species that traditionally has been associated with cutaneous leishmaniasis, has been isolated from bone marrow of five Desert Storm operation veterans, and from the lymph node of at least two additional cases. The clinical picture in many of these cases is atypical, and immunologic tests suggest that many additional systemic leishmanial infections have occurred.

Semi-quantitative PCR techniques were employed for detection of mRNA of various cytokines in bone marrow specimens from patients with visceral leishmaniasis (*Kala-azar*) in the Sudan. Relatively high levels of mRNA for IL-10 appear to be present in active cases, with a marked decrease in levels after treatment. These findings may help explain the antigen-specific anergy that occurs with this disease.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE. 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00161-15 LPD

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.E. Nash	Medical Officer	LPD,NIAID
Others:	M.M. Mowatt	Staff Fellow	LDP, NIAID
	D.H. Lujan	Visiting Fellow	LPD, NIAID
	L. Byrd	IRTA	LPD, NIAID
	G. Shearer		NCI
	P. Pimenta		LPD, NIAID

COOPERATING UNITS (if any) B. Gottstein, Institute of Parasitology, Univ. of Zurich, Switzerland

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Host-Parasite Relations Section,

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A system for classifying *Giardia lamblia* isolates was developed. Isolates fell into at least three major groups some of which differed greatly indicating these groups separated tens of millions of years ago. An adult mouse model of infection was developed. After an intense acute infection, trophozoites survive in very low numbers for months. A LIN-like Zn binding domain was identified in most variant- specific surface proteins and shown to bind Zn as well as other cationic metals. The biological significance of this is not known but it is possible that trophozoites bind intestinal Zn thereby preventing absorption and causing clinical or subclinical Zn deficient.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00162-16 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR	D.M. Dwyer	Supvy. Microbiol	LPD, NIAID
Others:	M.A. Alleman	IRTA Fellow	LPD, NIAID
	A. Debrabant	Visiting Fellow, FIC	LPD, NIAID
	J.A. Dvorak	Supvy. Microbiol	LPD, NIAID
	M. Gottlieb	Supvy. Microbiol	DMID, NIAID
	P.F.P. Pimenta	Guest Investigator	LPD, NIAID
	G.J. Russell	Visiting Fellow, FIC	LPD, NIAID
	C. McCarthy-Burke	Biologist	LPD, NIAID

COOPERATING UNITS *(if any)*
Depts. Biochem., Univ. Victoria (R.W. Olafson); CBER, DBB, FDA (M. Joshi and H. Nakhasi); Haskins Labs, Pace Univ. (C. Bacchi)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology & Cell Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	3.3	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The cell biology of both *Leishmania* and *Toxoplasma* are investigated as models of intracellular parasitism. Emphasis is placed on characterizing the biochemical and physiological functions of their surface membranes (SM) and secreted components toward defining the roles of these constituents in parasite survival and development.

Both *Leishmania* and *Crithidia* were shown to methylate surface membrane (SM) proteins in response to environmental stress. Cumulative quantitative data indicate that these organisms can adapt to environmental changes using methylation as a means to rapidly modify/regulate SM protein functions *in situ*. Over 800 nucleotides of a genomic clone of an *L. donovani* secretory acid phosphatase has been sequenced, and it is being used as a probe to obtain a full length cDNA copy of this gene. Experiments are also in progress toward cloning the gene for the unique trypanosomatid SM enzyme, 3'-nucleotidase. Methods were devised for the continuous *in vitro* cultivation of large quantities of infectious amastigotes from *L. donovani* and five other *Leishmania* species. These are being characterized with regard to their cell biology, SM-biochemistry and physiology. Further, at the molecular level, genes uniquely expressed by these amastigotes are being used as probes to study parasite gene regulated differentiation and development. In addition, studies were initiated concerning the functional SM biochemistry of *Toxoplasma gondii.*

The current results are of relevance toward the development of improved diagnostic, chemotherapeutic and immunoprophylactic agents against these important human pathogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00197-13 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Immunoregulation and immune recognition in filariasis and non-filarial diseases.

PRINCIPAL INVESTIGATOR	T.B. Nutman	Senior Investigator	LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	L. Elson	Visiting Fellow	LPD, NIAID
	S. Mollis	HHMI Rsrch Schlar	LPD, NIAID
	P. Zimmerman	NRC Fellow	LPD, NIAID
	S. Shaw	Senior Investigator	EIB, NCI

COOPERATING UNITS *(if any)*
Department of Allergy and Clinical Immunology, Univ. of Montreal, Montreal, Canada (G. Delespesse); Dept. of Clinical Investigation, Hospital Vozandes, Quito, Ecuador (Dr. R. Guderian), Univ. National de Benin (Dr. Massouboudgi), Anna Univ., Madras India (Dr. K. Jayaraman)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Clinical Parasitology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.2	0.8

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The purpose of this project is to delineate the mechanisms involved in regulating the humoral and cellular responses in patients with filariasis and other disease states. Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by showing this anergy to be a result of a diminished frequency of proliferating cells to parasite antigen and of the production of the antiproliferative cytokines, IL-10 and TGF-β. Filter immunoplaque assays for the major cytokines have been developed and used to demonstrate that in helminth infections of humans there is an expansion of Th2 CD4+ cells, the phenotypes of which are currently being delineated. *In vitro* models of parasite-antigen driven antibody production along with recombinant cytokines and neutralizing antibodies to them have been used to understand the signals necessary to regulate antibody production in response to parasite antigen.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00246-10 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Studies of the Genome and Surface of *Schistosoma mansoni*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Section Head	LPD, NIAID
Others:	I. Oswald	Special Volunteer	LPD, NIAID
	M. Williams	Special Volunteer	LPD, NIAID
	S.L. James	Program Officer	MIDP, NIAID
	T. Wynn	IRTA Fellow	LPD, NIAID
	J. Actor	IRTA Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*
Molecular Vaccines, Inc., Gaithersburg, MD (C.K. Stover, M. Hanson)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to characterize schistosome molecules relevant to immunity and pathogenesis, to analyze the molecular basis of physiologically important parasite processes which could serve as targets for intervention, and to develop and test recombinant vaccines. Progress was achieved in the following areas:

A. Construction of elastase and glutathione S-transferase BCG vectors

Recombinant BCG vectors expressing either elastase or glutathione S-transferase were constructed as candidate vaccines for preventing schistosome infection.

B. Development and testing of protective T cell lines and clones

A T cell line and clone were developed which transfer protection against challenge infection. The cytokine production patterns of the cells were characterized, and a study of their antigenic specificities initiated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00251-11 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR	A. Sher	Section Head	LPD, NIAID
Others:	I. Oswald	Special Volunteer	LPD, NIAID
	M. Williams	Special Volunteer	LPD, NIAID
	J. Actor	IRTA Fellow	LPD, NIAID
	T. Wynn	IRTA Fellow	LPD, NIAID
	A. W. Cheever	Assistant Chief	LPD, NIAID
	S. L. James	Program Officer	MIDP, IRP, NIAID

COOPERATING UNITS *(If any)*
Others, from above: J. Berzofsky, Section Head, MEI, NCI; R. Seder, Staff Fellow, LI, NIAID; W. Paul, Laboratory Chief, LI, NIAID.

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The aim of this project is to study mechanisms of immunity, immunoregulation, immune evasion, and immunopathology in schistosomiasis with the ultimate goals of developing an experimental vaccine suitable for human trials and understanding the pathogenesis of disease.

In work completed this year, the mechanism by which IL-10 inhibits macrophage-mediated killing of schistosomula was defined and shown to be due to suppression of TNF-α synthesis. In addition, IL-10 was shown to synergize with TGF-β and IL-4 in the inhibition of both larval killing and macrophage production of nitrogen oxide metabolites.

An analysis by PCR of cytokine genes expressed in the lungs of mice undergoing schistosome egg granuloma formation revealed a central role for IL-4, IL-2, and IFN-γ in this process. In related studies, it was discovered that Fcε-positive non-B, non-T cells were the major source of antigen-stimulated IL-4 synthesis in the spleens of infected mice.

Finally, schistosome-infected mice were shown to be defective in their ability to clear infections with a recombinant vaccinia construct expressing the GP-160 envelope protein of HIV-1. This decrease in viral resistance correlated with defects in the Th1 cytokine and CTL response to the virus.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-604-830

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00253-11 LPD

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Immunological Responses to Filarial Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	T.B. Nutman	Senior Investigator	LPD, NIAID
	N. Raghavan	Fogarty Fellow	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	L. Elson	Visiting Fellow	LPD, NIAID

See below

COOPERATING UNITS *(if any)* Indian Cncl of Med Res, Madras, India (S Tripathy, V Kumaraswami); Anna Univ, Madras, India (K Jayaraman); CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Coutinho); U. of Alabama (D.O. Freedman); Hospital Vozandes, Quito, Ecuador, (R. Guderian)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.2	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

J. McCarthy	Visiting Associate	LPD, NIAID
S.A. Williams	IPA	LPD, NIAID
F.A. Neva	Chief	LPD, NIAID

The purpose of this project is to define the humoral and cellular immune responses that relate to immunodiagnosis, immunopathology, and protective immunity of patients with lymphatic filariasis, onchocerciasis, and loiasis.

Serodiagnosis of new or prepatent onchocerciasis infections has been made feasible through the use of a purified recombinant onchocercal antigen (OV16). Serologic assays for filarial infection based on IgG4 antibody detection have also been shown to have enhanced specificity, but such antibodies require 6-9 months to develop despite the strong stimulation of chronic helminth infection. Molecular screening has identified recombinant antigens that provide for specific diagnosis of lymphatic filariasis.

The nature of the renal abnormalities in lymphatic filariasis has been examined and found to be expressed as hematuria with or without proteinuria. The enhancement of the hematuria during treatment and its resolution after therapy points to immune complex deposition as the underlying mechansism.

Populations with bancroftian filariasis or onchocerciasis have been examined to define immunologic parameters that distinguish "naturally immune" from infected individuals. A 43kD protein from infective larvae that may be a protective immunogen for bancroftian filariasis has been cloned and sequenced. Similar studies in onchocerciasis and in another focus of lymphatic filariasis have also been initiated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00255-11 LPD

PERIOD COVERED October 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Immunologic Responses to Non-Filarial Parasitic Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	T.B. Nutman	Senior Investigator	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	N. Raghavan	Visiting Scientist	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID

COOPERATING UNITS *(if any)*
CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Rochas)

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Host-Parasite Relations

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The tropical pulmonary eosinophilia syndrome (TPE) is generally caused by filarial parasites, but recent evidence indicates that strongyloides or ascaris infections may also induce a syndrome clinically indistinguishable from TPE. Detailed immunologic analysis is underway to define serodiagnostic means to differentiate among these clinical syndromes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH	PROJECT NUMBER Z01 AI 00256-11 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Developmental Biology of Leishmania Promastigotes

PRINCIPAL INVESTIGATOR	D. Sacks	Senior Investigator	LPD, NIAID
Others:	P. Pimenta	Guest Researcher	LPD, NIAID
	L. Carrera	Guest Researcher	LPD, NIAID
	E. Saraiva	Guest Researcher	LPD, NIAID

COOPERATING UNITS *(if any)*
Dr. Sam Turco, Dept. of Biochemistry, Univ. of Kentucky, Dr. Malcolm McConville, Dept. of Biochemistry, Univ. of Dundee, Scotland, Dr. Ed Rowton, Walter Reed Army

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

During growth within the sand fly and within axenic culture, *Leishmania* promastigotes undergo differentiation from a dividing non-infective stage to a resting infective or metacyclic stage which is uniquely adapted for life in the vertebrate. The culture conditions which promote differentiation of parasites to an infective stage have now been optimized for various strains. This development is accompanied by a substantial modification of the surface lipophosphoglycan (LPG) which is the major surface glycoconjugate of these cells. The developmental modifications include an elongation of the molecule due to a 2-3 fold increase in the number of phosphorylated oligosaccharated units expressed, and a change in the composition of some of these units. In the case of *L. major* and L. donovani the structural polymorphism is expressed as a loss of terminal galactose residues on the LPG. Related structural changes have now been confirmed for *L. amazonensis* and *L. infantum*. The substitution or capping of terminally exposed sugars with other sugar residues was shown to be the molecular mechnism underlying the attachment and release of promastigotes from midgut epithelial cells during their development in the fly. The specific oligosaccharide units which mediate the binding have now been identified. The species specificity of these terminal sugars appears to influence the species specificity of vectorial capacity observed in nature. LPG has been successfully used to probe for the lectin like receptors in the sand fly gut. In related studies, it was found that the presence of anti-LPG antibodies within an infective bloodmeal inhibited the development of transmissible infections within the sand fly midgut. This inhibition was only apparent when the bloodmeal also contained anti-leishmanial antibodies present in chronic infection sera.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-854-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00257-11 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR	F. A. Neva	Chief	LPD, NIAID
Others:	C. Brown	Expert	LIR, NIAID
	E. Denkers	IRTA Fellow	LPD, NIAID
	T. Nutman	Senior Investigator	LPD, NIAID
	N. Raghavan	Guest Investigator	LDP, NIAID
	E. Ottesen	Senior Investigator	LDP, NIAID
	T. Nash	Senior Investigator	LDP, NIAID
	D. E. McFarlin	Chief	NI Branch, NINDS

COOPERATING UNITS *(If any)*
Bioqual, Inc., Rockville (T. Moskal); U of the West Indies, Jamaica (Prof. O. Morgan, R. Robinson, & S. Terry); Project SIDA, Kinshasa, Zaire (J. Perriens); U of Mass., Dept. of Family Medicine (P. Gann)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.9	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

This project deals with both laboratory and clinical aspects of strongyloidiasis; the former with analysis and characterization of parasite antigens and immune response to them, and the latter with investigations of patients infected with this parasite. The parasite is itself unique because of certain biologic properties (such as absence of a parasitic male worm, free-living stage and internal auto-infection) and because of the fact that it can produce a serious, even fatal disease in certain immunosuppressive states.

Since we have experienced problems in purification of the larval protease, attention will be focused upon other antigens that can be identified which react with IgE in pooled patients' serum. A cDNA library has been prepared from 3rd stage larvae of *S. stercoralis*, and several clones have been identified.

Clinical studies, except for providing laboratory assistance in diagnosis of suspect cases, have been focused upon examining the IL-4 and IL-5 cytokine responses of selected cases to mitogenic and antigenic stimuli *in vitro*. The code has not yet been broken to see the results of a comparative treatment trial of strongyloidiasis with Thiabendazole vs. Ivermectin. This was carried out in collaboration with investigators in Lowell, Massachusetts, on Cambodian refugees found infected with the parasite.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00347-10-LPD

PERIOD COVERED October 1, 1991 - September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.W. Cheever	Head, Host-Parasite Relations	LPD, NIAID
Others:	D. Abrams	Bio. Lab. Tech.	LPD, NIAID
	T. Cox	Bio. Lab. Tech.	LPD, NIAID
	I. Eltoum	Visiting Fellow	LPD, NIAID
	A. Sher	Head, Immunology Sect.	LPD, NIAID

COOPERATING UNITS *(if any)*

Department of Medicine, USUHS (Fred Finkelman) and USDA (Joseph Urban). Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil (Zilton Andrade).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION NIAID, NIH Bethesda, Md 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Morbidity in schistosome infected individuals is caused mainly by the immune response of the host to schistosome eggs deposited in the tissues. In chronic schistosomiasis fibrotic sequellae to the inflammatory reaction to the eggs are responsible for most clinical disease.

Hepatic fibrosis and the granulomatous response to eggs of schistosome species pathogenic for man are studied in mice in relation to parasitologic parameters of infection. Cytokines play an important role in the genesis and regulation of the size of circumoval granulomas and in the fibrosis associated with them. Treatment of mice with antibodies to IL-2 or IL-4 markedly decreased hepatic fibrosis in *S. mansoni* and *S. japonicum* infected mice while having trivial effects on the size of granulomas around the schistosome eggs.

Our results suggest that Th2 type cell responses are important for the fibrotic responses to schistosome eggs and, by analogy, perhaps for other hypersensitivity reactions in which "delayed hypersensitivity" is important.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER
Z01 AI 00350-10 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR	T. E. Nash	Medical Officer	LPD, NIAID
Others:	M. M. Mowatt	Staff Fellow	LPD, NIAID
	J. Moss		NHLBI
	J. Murtagh		NHLBI
	A. Geber		LCI, NIAID
	J. Weiss		Roche Diagnostics
	R. Adam		University of Arizona

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Large differences among *Giardia* isolates were noted. 18S rDNA sequence differerences were detected among isolates but were identical among the same *Giardia* Group. Comparison of the triosphophate isomerase genes of two isolates known to differ from each other by other criteria revealed a 19% sequence difference which resulted in a 9% amino acid divergence. A transcript was identified, sequenced and characterized. It was transcribed in only one of three Groups of *Giardia* and therefore is a unique marker of this group. The genomic organization of the VSP genes can differ among isolates.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00439-08 LPD

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical and Therapeutic Studies in Human Filariasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	T.B. Nutman	Senior Investigator	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID

COOPERATING UNITS *(if any)* Indian Council of Medical Research, Madras, India (S.P. Tripathy, V. Kumaraswami, R. Prabhakar); MGR Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office (C. Waterman); Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil (A, **See below**

LAB/BRANCH Laboratory of Parasitic Diseases

SECTION Clinical Parasitology Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
14	1.0	0.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Coutinho, G. Dreyer); Dept of Health, Cotonou, Benin (A. Massougbodji), Department of Medicine, Univ. of Iowa, Iowa City, Iowa (A. Klion).

Ivermectin has been shown in trials in both South India and elsewhere to be very effective in clearing microfilaremia in patients with bancroftian filariasis. Current studies in South India and Brazil (coordinated with trials elsewhere in the world) are focused on developing a regimen to optimize the duration of microfilarial clearance.

Recent investigation has indicated that the Tropical Pulmonary Eosinophilia syndrome can be caused by nonfilarial parasites as well as by filariae. Work to differentiate these two pulmonary eosinophilic conditions and develop appropriate therapy is underway using tools of molecular characterization.

Loiasis in expatriate visitors to endemic areas has been characterized as having marked clinical and immunologic hyperresponsiveness to the filarial parasite; predictors of relapse and response to definitive therapy have been analyzed and found only to relate to the strain of infective parasite. Because standard therapy for loiasis in heavily infected individuals is quite dangerous, a trial of albendazole for loiasis has been completed and appears to be safe and efficacious.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00487-06 LPD

PERIOD COVERED October 1, 1991 - September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Quantitative Parasitology of Schistosome Infections.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.W. Cheever,	Chief, Host-Parasite Relations Section	LPD, NIAID
Others:	D. Abrams	Bio. Lab. Tech.	LPD, NIAID
	T. Cox	Bio. Lab. Tech.	LPD, NIAID
	J. Mosimann		OSI, OD
	Z. Andrade	Fogarty Scholar	
	J. Actor	Immunology section	LPD, NIAID

COOPERATING UNITS *(if any)*
Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil (Zilton Andrade). Biomedical Research Institute, Rockville, MD (Fred Lewis).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Morbidity in schistosomiasis is caused by the host response to schistosome eggs which a deposited in the venous system and carried to the tissues. Pathology is proportional to the number of eggs laid and it is thus important to understand factors underlying worm fecundity and the extent to which fecundity is reflected by eggs passed in the feces, the measurable indicator of infection intensity in humans.

The rate of destruction of *S. mansoni* or *S. japonicum* eggs in the tissues of mice has proved to be so slow as to make it a negligible factor in calculating the fecundity of schistosomes. The fecundity (essentially = to eggs/day passed in the feces + eggs/day accumulated in the tissues) of *S. mansoni* in mice infected with a single pair of worms averages about 330 eggs/day/female and eggs passed in the feces reflect the intensity of egg laying in individual mice over a 1 year period of infection. *S. japonicum* lays over 2000 eggs/day/female early in infection but the rate of egg laying decreased with increased duration of infection to less than 1000 eggs/day/female 1 year after infection. The number of eggs in the feces decreases in parallel to the decrease in total egg laying. The fecundity of *S. mansoni* but not *S. japonicum* is decreased in SCID mice. *S. mansoni* frequently fails to mature in nude mice but worms which mature produce normal numbers of eggs.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH**	PROJECT NUMBER Z01 AI 00494-06 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Analysis of T Cell Responses in Human Leishmaniasis

PRINCIPAL INVESTIGATOR	D. Sacks	Senior Investigator	LPD, NIAID
Others:	A. Cooper	Fogarty Fellow	LPD, NIAID
	C. Karp	Guest Researcher	LPD, NIAID
	F. Neva	Chief	LPD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Although there is strong evidence that control and resolution of human leishmanial infections depends primarily on T cell derived lymphokine mediated activation of parasite infected macrophages, less is known about the nature of the responding cell type(s) which are protective or the Ag(s) that elicit these cells to respond. Studies using whole soluble Ag ("dead Ag") preparations show that patients respond to a wide range of leishmanial Ag. The objective of recent studies has been to characterise the response of T cells from patients with healing or healed cutaneous or mucosal infections to Ag expressed by or derived from actively infected autologous monocytes ("live Ag"). Unfractionated T cells proliferated and produced IFN-γ in response to both "live" and "dead" Ag. Depletion of CD4+ T cells resulted in the loss of proliferative and IFN-γ responses to both "live" and "dead" Ag. The effect of CD8 depletion, was variable, and not limited to the cells stimulated by infected monocytes. Expansion of T cells specific for "live" antigens using amastigote infected cells followed by re-stimulation with HPLC fractionated soluble antigens revealed that a diversity of antigens are associated with infected monocytes. There may, however, be quantitative differences in the expression of certain antigens since "live" versus "dead" antigens could distinguish responses between MCL and LCL patients.

The immune responses in patients with non-healing, visceral forms of disease have continued to be a focus of study. The critical immunological feature of visceral leishmaniasis is the complete absence of cell mediated immunity to leishmanial antigens. Patients have been shown to have negative intradermal skin tests to *leishmania*, absent lymphocyte blastogenesis, and decreased IL-2 and IFN-g production in response to parasite antigens. Despite this unresponsiveness, there is evidence that antigen-specific T cells are present in *kala-azar* patients. Visceral leishmaniasis is accompanied by high titres of antileishmanial antibodies, most of which are likely to be T-cell dependent. Additionally, T-cells are required are likely to be required for successful chemotherapy of visceral disease. Mouse models of cytokine-mediated lymphocyte functional regulation provide a theoretical framework for the present investigation of the antigen-specific anergy of *kala-azar*. For a descriptive study of cytokine patterns in *kala-azar*, we sought to maximize assay sensitivity by using semi-quantitative reverse-transcriptase PCR techniques to analyze cytokine mRNA extracted from lesional tissue -- bone marrow. In preliminary data we provide evidence that IL-10 mRNA is present at relatively high levels in active *kala-azar*, being at or below the limits of detectability after treatment, and absent in uninfected controls.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-654-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00512-05 LPD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Molecular Definition Of Filarial Antigens

PRINCIPAL INVESTIGATOR	T. B. Nutman	Senior Investigator	LPD, NIAID
Others.	N. Raghavan	Visiting Scientist	LPD, NIAID
	J. McCarthy	Visiting Associate	LPD, NIAID
	L. Elson	Visiting Fellow, FIC	LPD, NIAID
	S. Mollis	HHMI Rsrch Scholar	LPD, NIAID
	S. Williams	IPA	LPD, NIAID
	E. A. Ottesen	Senior Investigator	LPD, NIAID

COOPERATING UNITS *(if any)*
New England Biolabs, Beverly, Mass (F. Perler, L.McReynolds, C. Maina), Department of Geographic Medicine, University of Alabama, Birmingham (D. Freedman), Swiss Tropical Institute (E. Lobos)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Clinical Parasitology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The objectives of this project are to define and generate filarial proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related filarial species.

Recombinant antigens and probes have been identified that: a) encode immunoreactive and potentially protective molecules of *W. bancrofti*; b) can distinguish among related filarial species; c) identify repeated segments of either the *W. bancrofti* genome or that of *Loa loa*; d) may be of potential diagnostic importance; and e) induce immediate hypersensitivity type responses in lymphatic filarial syndromes.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE. 1991 0-844-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00579-03 LPD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Immune Regulation in Toxoplasmosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Senior Investigator	LPD, NIAID
Others:	R. Gazzinelli	Staff Fellow	LPD, NIAID
	E. Denkers	IRTA Fellow	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID
	D. Margulies	Senior Investigator	LI, NIAID
	H.C. Morse	Chief	LIP, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The overall aim of this project is to analyze the immune response to *Toxoplasma gondi* in order to define which cellular immune components and parasite target antigens are involved in the control of infection and its breakdown in immunocompromised hosts.

Progress was made this year in the following areas:

A. Analysis of Class I MHC presentation pathways and CD8+ cell target molecules utilized by *T. gondii*. Bone marrow macrophages were shown to process soluble *T. gondii* antigens for presentation to CD8+ T cells by an endogenous pathway. A preliminary analysis of the processed parasite proteins and CTL target peptide epitopes was performed.

B. Role of cytokines in down-regulation of macrophage-mediated killing of *T. gondii* and *T. cruzi*. IL-10, IL-4 and TGF-β were shown to down-regulate the intracellular killing of *T. gondii* by activated macrophage through the inhibition of nitrogen oxide production. Parallel results were found with a related intracellular parasite, *Trypanosoma cruzi*.

C. Role of NK cells in the cytokine response to *T. gondii*. *T. gondii* was shown to trigger the T lymphocyte-independent production of IFN-γ by NK cells. SCID mice depleted of NK cells were shown to be more susceptible to infection, and NK cells were shown to replace CD8+ cells as the primary effectors of immunity in vaccinated β2-microglobulin-deficient mice.

U.S. GOVERNMENT PRINTING OFFICE: 1991 [illegible]

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1992 Annual Report
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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1991 to September 30, 1992

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD this past year:

Arrivals:

Dr. Kim Hasenkrug, converted to Staff Fellow (previously an IRTA Fellow in LPVD) working with Dr. Bruce Chesebro.

Ms. Shelly Robertson, Biologist, from Washington State University, Pullman, Washington, working with Dr. John Portis.

Departures:

Dr. Dahn Clemens, IRTA Fellow, going to work at the Department of Medicine, Veterans Administration Medical Center, Omaha, Nebraska.

Dr. Raymond Monk, Senior Staff Fellow, plans to obtain further law enforcement education to eventually pursue forensic research.

Dr. Brian Dannemann, going into private practice in infectious diseases with the Central Utah Medical Clinic, Provo, Utah.

Summer students were Bradley Berry from Caroll College, Helena, Montana; John R. Courchesne from University of Montana, Missoula, Montana; Dorothy Hernandez of Oberlin College, Oberlin, Ohio; Mark Huhtanen of Darby High School and Montana State University, Bozeman, Montana; Michelle Loftis from the University of Nebraska, Omaha, Nebraska; James Nolz from University of Nevada, Reno, Reno, Nevada; Shelly Robertson of Washington State University, Pullman, Washington; Shannon Waliser of Caroll College, Helena, Montana; and Jessica Walker from Swarthmore College, Swarthmore, Pennsylvania.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1991 to September 30, 1992

OVERVIEW OF RESEARCH IN LPVD

Research at LPVD is aimed at understanding mechanisms of virus persistence and latency with particular emphasis on immune surveillance by the host and virus evasion of host defenses operating in different systems.

In the case of murine retroviruses, the following areas are being studied:

Characterization of host genes and immune mechanisms involved in recovery from virus-induced leukemia, use of vaccinia-retrovirus recombinants to induce protective immunity, use of retrovirus expression vector systems to study cell tropism of infection and direct expression of exogenous genes to different cell types. (Chesebro, Hasenkrug, Dannemann)

Study of degenerative central nervous system disease induced by mouse retroviruses including characterization of viral gene sequences influencing cell tropism, disease kinetics and pathogenic events at the light and electron microscopic level. (Portis, Lynch, M. Czub, S. Czub)

Study of the specificity of generation of recombinant retroviruses by interactions between exogenous viruses and endogenous viral sequences. Characterization of the role of recombinant retroviruses in leukemogenesis and autoimmunity. (Evans, Monk)

Studies are also in progress on human and equine lentiviruses (HIV and EIAV). We have developed a focal *in vitro* infectivity assay for these viruses. Cell surface receptors and other cellular factors influencing cell tropism of infection are being studied. AIDS patient HIV isolates with unusual cell tropism are being examined by PCR amplification and cloning to determine influence of specific viral gene sequences on cell tropism. Pseudotypes of HIV genomes with envelope proteins of other human or murine retroviruses are being studied for patterns of altered tropism *in vitro* and *in vivo.* (Chesebro, Maury)

Other virus systems being studied include rabies virus in mice, Aleutian disease virus of mink, and the mouse-adapted strain of the scrapie agent.

In the murine rabies model, host genes have been found to influence ability of mice to recover from infection. In some mouse strains, recovery can occur even after CNS infection and clinical symptoms have developed. The immune mechanisms of this recovery are the main focus of study. (Lodmell)

Aleutian disease virus (ADV), a parvovirus, has been investigated in adult mink as an infectious model of the human disease systemic lupus erythematosus and in newborn mink as an infectious model for the hyaline membrane-associated respiratory disease of newborn humans. In the mink there are susceptibility and resistance genes in the host as well as virus strains which vary in virulence and host tropism. Current efforts at LPVD have resulted in molecular cloning of several virus strains and DNA sequencing is underway to determine which genes control virulence. More recently, strand-specific ADV probes have been used in *in situ* hybridization to distinguish cells replicating ADV from cells where ADV virions accumulate during disease. In addition, monoclonal antibodies specific for individual virus strains have been developed and are being used to characterize sites of virus replication and virus-antibody complex deposition *in vivo.* (Kanno, Clemens, Bloom)

Epidemiology and pathology of the scrapie agent were studied by the slow virus group at RML for many years. So far we have cloned and sequenced a cDNA copy of the mRNA encoding the mouse scrapie "prion" protein. This gene was found to be expressed in both infected and uninfected animals, suggesting that this protein is not virus-encoded, but instead may be a normal brain protein involved in amyloid deposition during scrapie pathogenesis. Recently emphasis has switched to development of *in vitro* culture systems for scrapie. Neuroblastoma cell lines persistently carrying the transmissible agent have been developed, and cell cloning has yielded cultures where almost all cells are "infected". Current efforts are directed towards biochemical characterization of the transmissible agent in these cells. (Race, Chesebro, Caughey, Priola, and Doh-Ura)

Amyloid has also been the focus of studies in a nonviral model in hamsters. A hamster serum protein under sex hormonal control was identified and found to be a component of spontaneous and induced amyloid in hamsters. During experimental hormonal manipulation of hamster serum proteins, estrogens were found to induce hepatomas in hamsters. Possible role of oncogene- or growth factor receptor-indicated mechanisms of tumorigenesis are being studied. (Coe, Satoh)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00072-21 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: D. L. Lodmell Scientist Director LPVD, NIAID

Other:

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The principal objectives of this research project are two-fold: 1) to determine host and viral factors that influence the genetically controlled resistance of inbred mouse strains to street rabies virus (SRV) and 2) to determine the mechanism(s) by which recombinant vaccines expressing different structural proteins of the rabies virus protect mice against lethal infection.

In a continuation of our cytotoxic T lymphocyte (CTL) studies, we have determined that CD8+ T cells had no effect on the spontaneous recovery of BALB/c mice from central nervous system (CNS) infection whether or not anti-CD8 Mab was administered throughout the disease course or only during the interval of recovery from CNS infection. In contrast, depletion of CD4+ T cells during these same intervals resulted in 100% mortality which correlated with a complete depression of virus-specific neutralizing antibody production.

In contrast to euthymic mice, SRV-infected T-lymphocyte-deficient (nude) mice did not develop hindlimb paralysis prior to death. We documented the role of T lymphocytes in rabies virus-associated paralysis by showing that recipient nude mice which received spleen cells from normal immunocompetent euthymic mice became paralyzed following SRV infection. Depletion of T cells from the donor spleen suspension prior to transfer abrogated the development of paralysis, but did not prevent the deaths of recipient animals. CNS inflammatory responses in nude mice immunologically reconstituted with normal spleen cells were characterized by diffuse cellular infiltrates in the parenchyma and extensive perivascular cuffing. Perivascular infiltrates included CD8+ and CD4+ T lymphocytes and Mac-1+ macrophage-microglial cells. Inflammatory cells in the parenchyma were limited to CD8+ lymphocytes and Mac-1+ cells. These observations indicate that paralysis of rabies-infected mice is dependent on T lymphocytes.

Studies with recombinant vaccines expressing either the rabies virus glycoprotein (G) or nucleoprotein (N) are continuing. *In vitro* and *in vivo* models to detect protective activity in sera of N vaccinated mice have been established. Although we are certain that protection of N-vaccinated mice is due to anti-N antibody, the mechanism of protection is unclear. Our results suggest that the N protein, because of its remarkable sequence conservation among N proteins of different rabies viruses, should be incorporated with the G protein in recombinant vaccines.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-884-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00074-20 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetically Controlled Mechanisms of Pathogenesis and Recovery in Friend Retrovirus-Induced

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	K. Hasenkrug	IRTA	LPVD, NIAID
	B. Dannemann	Research Associate	LPVD, NIAID

COOPERATING UNITS *(if any)*

M. Miyazawa	Department of Pathology, Tohoku University, Sendai, Japan
M. Robertson	Pastern Institute, Paris France

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This project contains studies aimed at elucidating genetically controlled host defense mechanisms involved in spontaneous recovery and protective immunity against Friend murine retrovirus-induced leukemia.

MHC genes play an important role both in spontaneous recovery and in protective immunity induced by vaccination. Our recent results show that point mutations in either the I-A^b gene or the D^b gene are sufficient to inhibit spontaneous recovery. The I-A^b gene acts as a typical immune response gene facilitating the T helper cell response to Friend virus envelope protein. The D^b gene appears to influence development of virus-specific cytotoxic T lymphocytes. These cells are strongly associated with the actual recovery process *in vivo*, and our current data indicates that they are specific for the viral envelope protein. The D^b haplotype may operate to facilitate recovery by selecting certain viral envelope peptides as targets for immune lysis.

Protective immunity can be induced in this system by inoculation with killed whole virus or purified viral envelope protein in adjuvant or recombinant vaccinia virus expressing the viral envelope protein. The I-A^b gene is important in mediating a successful response to immunization, but animals lacking this gene can be protected if immunization is done using a powerful adjuvant. Recent data indicates that some synthetic vaccines feasible for use in humans also work to potentiate successful immune protection in this model.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00085-15 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	H. Kanno	Visiting Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
Department of Pathology, Tohoku University School of Medicine, Sendai, Japan
Division of Virology, Department of Neuropharmacology, Scripps Research Institute, La Jolla, CA

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.5	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical IIDEA project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The scope of this project is to elucidate pathogenic mechanisms involved in infections of mink with Aleutian mink disease parvovirus (ADV).

In the past year we continued studies on the replication of ADV in macrophages. Expression and replication of the ADV genome was examined in primary peritoneal macrophage cultures derived from adult mink. A small percentage of macrophages infected with the pathogenic ADV-Utah 1, but not the cell culture adapted ADV-G, expressed ADV antigens following infection. Analysis of DNA prepared from ADV-Utah 1 infected macrophages revealed definite amplification of the ADV genome and the presence of ADV DNA replicative forms (RF), but the level of gene expression was too low to allow the definition of specific viral proteins by Western blot.

Several human monocyte cell lines (U-937 and THP-1) were also found to support ADV-Utah 1 expression and the expression could be modulated by treatment with an inducer of macrophage differentiation. Virus could not be serially propagated in U-937 cells, thus the infection was not fully permissive, but was restricted as is seen in vivo infections. The various replicative forms of ADV DNA were present, but the relative level of single stranded virion DNA appeared to be lower than in permissively infected cells. The 2 capsid proteins and the major nonstructural protein, NS-1, were present in infected U937 cells. The three size classes of viral mRNA were observed, but the 2.8 kb mRNA band in these cells could be resolved into 2 definite components, slightly different from permissively infected cells. This finding might reflect a variation in the expression of the smaller NS-2 protein between permissive and restricted ADV infections.

Mink lymph node cultures were found to express a bioactive IL-6, but the gene for mink IL-6 has not yet been cloned. In addition, U937 cells infected with ADV-Utah 1, but not ADV-G, elaborate human IL-6.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00086-15 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Portis	Medical Officer	LPVD, NIAID
Other:	William Lynch	Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
Dr. Lennert Mucke and Dr. Michael Oldstone, Scripps Clinic and Research Foundation, La Jolla CA.

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.0	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The retroviruses we study were isolated from wild mice and cause a non-inflammatory neurodegenerative disease in mice similar to that caused by the unconventional agents such as scrapie. We are using this animal model to study the pathogenesis of retrovirus-induced neurodegeneration. Viral sequences: We previously showed that viral sequences within the 5' leader sequence of the viral genome influence the level of CNS infection and, if the virus contains an *env* gene from a neurovirulent retrovirus, the length of the incubation period of neurodegenerative disease. This sequence appears to influence the kinetics of virus replication in peripheral organs. We speculated that the effect of this sequence on CNS infection involved the fact that the CNS is susceptible to infection only until the 12th postnatal day, after which it becomes resistant. Strong support for this hypothesis has come from embryo inoculation experiments. Neonatal inoculation of the slowly replicating virus, 15-1, results in a low level CNS infection and very long incubation period of ≥6mo. However, embryo inoculation on E9 resulted in high level CNS infection and rapid disease being seen within 3 weeks postnatally. Thus, it appeared that the only constraint to CNS infection by this virus was the rate of virus replication, giving the virus a "head-start" overcame this constraint. Host Factors: We have found that whereas virus infection of the central nervous system is necessary, it is not sufficient for the expression of neurodegeneration. A postnatal developmental event(s) is required for the induction of spongiform degeneration. This event appears to occur 8-10 days postnatally. Currently the nature of this developmental event is uncertain. Mechanism of neuronal cytopathology: The role of the glial infection in this disease has been tested *in vivo* through selective infection of glial cells within susceptible regions of the CNS. No evidence of neuronal cytopathology was observed suggesting that glial infection alone is not sufficient for the induction of this neuronal cytopathology. A temporal study of the evolution of spongiform lesions has revealed that lesions appear in all affected areas of the CNS simultaneously within a narrow window of time ca. 10 days postnatally. Furthermore, there is a close temporal relationship between the first appearance of vacuolar neuronal degeneration and the expression of viral protein by premigration cerebellar granule neurons, suggesting that infection of the cerebellar cortex may play a central role in disease pathogenesis. These studies are providing further insight into the mechanisms by which retroviruses damage neurons and are beginning to reveal the ways in which retroviruses can adversely affect the developing brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00262-11 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Pentraxins in Acute and Chronic Pathology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. E. Coe	Medical Officer	LPVD, NIAID
Other:	B. Caughey	Senior Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
Drs. B. Canquilhem & P. Pevet, Strasbourg, France; Dr. B. Dowton, Wash. Univ. Med. School, St. Louis, MO; Dr. K. Ishak, AFIP, Washington, D.C.; Dr. U. Nilsson, Uppsala, Sweden; Dr. Mortensen, OSU, Columbus, OH; Dr. D. Johnson, U. Kansas Med. Center, Kansas City; Dr. Gary Nelsestuen, U. Minn., St. Paul, Dr. J. Ward, NCI, Ft. Dietrick, MD; Dr. T. Duclos, U. New Mex., Albuquerque, NM; Dr. H. Gewurtz, Rush Med. Center, Chicago, IL; W.S. Caughey & A Dong, Colo. State U., Fort Collins, CO

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION
NIAID, NIH, Bethesda, MD 20892

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

We have been studying the endocrine and cytokine control of a protein synthesized in liver of Syrian hamster. This protein is homologous to two human proteins, C. reactive protein (CRP) and amyloid P component (AP), and shares 50-70% (respectively) identical amino acid sequence. These proteins also share a similar molecular configuration which is that of an oligomer of 5 identical monomer proteins noncovalently assembled as a cyclic pentamer. This family of proteins is called pentraxin, and the pentraxin of Syrian hamster is called female protein (FP). FP shares similar functional properties with CRP and AP such as Ca++ dependant Phosphoryl-choline and galactan binding, complement fixation and acute phase responsiveness. Pentraxins are widely expressed in nature, and are found even in very primitive animals. The conservative evolution of this protein family and its ubiquitous expression suggests that pentraxins are biologically important. However, a critical role for these proteins has not been found yet. In hamsters, females have 200-300 fold more FP in serum than males, and this concentration difference reflects the different rate of hepatic synthesis by males and females. This unusual endocrine control suggests that FP may have some unusual function in the hamster which could provide information about the general function of all pentraxins. We have not found any beneficial effect from FP, and in fact high synthesis rates of FP appears to result in a shortened longevity of the female hamster. In Syrian hamsters, females normally die at an earlier age than males, (in contrast to all other mammals) and female hamsters generally die because of amyloidosis. Similar to AP in human amyloid, FP of Syrian hamsters is a constituent of amyloid, and we have shown that high serum levels of FP appear to have a primary role in the induction of amyloidosis in hamsters. The endocrine control of FP synthesis is different in other hamsters. Thus, serum FP concentration is lower in Turkish hamsters and this species, which is very closely related to Syrian hamster does not acquire amyloidosis. Turkish hamsters appear to have an FP with identical amino acid sequence to Syrian hamster FP, although serum FP levels in females are about 1/10 that found in Syrian hamsters. Control of FP synthesis in Armenian hamsters is especially different from Syrian hamster because estrogen actually down regulates Armenian FP synthesis. Exogenous estrogen also is extraordinarily toxic to livers of Armenian hamsters and chronic estrogen administration initiates in the appearance of hepatic neoplasms. Estrogen induced hepatotoxicity (cholestatic jaundice of pregnancy) and hepatic cancer are known to occur in humans, but there are no comparable animal models to study these phenomena.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00263-11 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	Dahn Clemens	IRTA	LPVD, NIAID

COOPERATING UNITS *(if any)*
Department of Veterinary Virology, Royal Danish Veterinary University, Copenhagen, Denmark
Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland
Department of Veterinary Microbiology, Iowa State University

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical IIDEA project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The purpose of this project is to relate structural features of the ADV genome to the ability of various virus isolates to replicate in culture and to cause disease in mink.

We have continued to study molecular clones chimeric for genomic segments between the nonpathogenic ADV-G and pathogenic ADV strains. By performing segmental exchanges and doing PCR mutagenesis, we have found that mutating a single glycine residue to aspartic acid in the capsid proteins gene abrogates the ability of an infectious ADV-G clone to replicate and produce infectious virus in cell culture. By comparing transfections with full length clones, we have found that the block to replication appears to be a failure to generate single stranded progeny DNA. None of the viruses rescued from full length clones cause disease in adult mink, suggesting that pathogenicity of ADV and its ability to replicate permissively in CRFK cells are linked in a negative fashion. Additional ADV-Utah 1 sequence information has been obtained which indicates substantial sequence variation not previously recognized.

Using prokaryotic expression systems, we demonstrated the existence of a nonstructural protein-2 (NS-2) for ADV, but have failed to identify a predicted gene product of the ADV RX mRNA (NS-3).

Recombinant vaccinia viruses expressing either both VP1 and VP2 or VP2 singly synthesize appropriately sized proteins; the proteins are transported to the nucleus and form empty virus particles. Thus VP1 is not required for particle assembly. The VP1/VP2 capsids, but not the VP2 only ones, can bind to ADV DNA replicative forms like bona fide ADV virions. The recombinant viruses stimulate anti-ADV antibody in mice, but appear to be poorly immunogenic in mink. A similar VP1 and VP2 segment has been introduced into a recombinant baculovirus, denoted AcADV, which also expresses both proteins and the proteins self-assemble into capsids.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-854-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 A1 00265-11 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. E. Race	Research Veterinarian	LPVD, NIAID
Other:	B. Caughey	Staff Fellow	LPVD, NIAID
	S. Priola	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS *(if any)*
Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN; Dr. Al Jenny, U.S.D.A., Ames, IA; Dr. Lyle Miller, Iowa State University; Dr. Randall Cutlip, U.S.D.A.; Dr. Michael Oldstone, Scripps Clinic, San Diego CA

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.2	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Scrapie is a spongiform encephalopathy of sheep and goats which can be transmitted experimentally to several other animal species. Similar diseases are recognized in cattle and humans. No etiologic agent has been identified. However, the proteinase K resistant form (PrP-res) of an endogenous protein designated prion protein (PrP) purities with infectivity and is important to disease pathogenesis.

We developed a sensitive assay for PrP-res and utilized it to diagnose scrapie in sheep. Analysis based on PrP-res detection was much more accurate and less subjective than the currently used method of diagnosis based on the microscopic evaluation of brain. We also showed that PrP-res analysis of spleen or lymph node was nearly as accurate as analysis of brain. We are determining if PrP-res accumulates prior to clinical disease in lymph node. If so, an ante mortem test for infection may for the first time be possible. We are also using PrP-res analyses to test tissues from cattle in order to determine if spongiform encephalopathy currently exists in U.S. cattle and, thereby, whether an epidemic similar to BSE in Great Britain is possible in the U.S.A. PrP-res analysis should also be relevant for diagnosis of the human disease counterparts.

We are also using a scrapie-susceptible mouse neuroblastoma cell line (MNB) to study the influence which specific PrP gene sequences have on interspecies transmission of spongiform encephalopathies. To do so, we have expressed various mouse-hamster PrP constructs in the scrapie-infected cells and are now analyzing them in mice and hamsters to determine if species tropism has been altered. Similar studies could tell us if BSE represents a risk to humans.

In addition, the MNB cells as well as a pluripotent embryonal carcinoma cell line (P-19) are being used to study the normal function of the PrP protein as well as factors which might account for the biochemical changes which lead to the conversion of the endogenous PrP protein to the PrP-res form.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-884-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00266-11 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. H. Evans	Chemist	LPVD, NIAID
Other:	R. J. Monk	Senior Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
Dr. Seth Pincus, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton, MT
Dr. John Portis, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories,

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Genetic variation is a hallmark of retrovirus infection and occurs in avian, murine and human retroviruses such as HIV. The major focus of this project is the study of genetic variation in retroviruses and the effect of such variation on virus infection and disease. Studies currently underway in the laboratory include the determination of the point mutation rate of retroviruses; the polymorphism of recombinant host range retrovirus variants; pseudotyping as a result of mixed retrovirus infections; and the treatment of retrovirus infections with immunotoxins reactive with retrovirus-coded antigens.

A. Reported estimates of mutation rates of retroviruses suggest that progeny retroviruses sustain several point mutations during a replication cycle. In contrast we have determined that most progeny murine retroviruses do not sustain any point mutations during a single cycle. Studies are underway to compare the point mutation rate of viable progeny viruses to that of proviruses in the absence of selection for viability.

B. Inoculation of many murine retroviruses in mice results in the generation of host range variants by recombination of the inoculated virus with endogenous retroviral genes. We have identified two major antigenic subgroups of these variants. The subgroups appear to be derived from distinct endogenous sequences and differ with respect to their in vitro cell tropisms. We are currently constructing chimeric viruses between viruses of the two major subgroups to map the genetic determinants of their properties.

C. The generation of host range variants in retrovirus-infected mice results in a mixed retrovirus infection which may give rise to viral pseudotyping. We have found this to be the case in mice infected with the Moloney lymphocytic leukemia virus. Moreover, the pseudotyping appears to be a dynamic phenomenon which varies in different organs, changes during the course of disease and may influence the spread of infection of the target organ by host range variants. Continuing studies will examine the extent of virus spread and the cell-types infected by the host range variants.

D. Ricin A-chain immunotoxins constructed using antibodies reactive with murine retrovirus-coded proteins have been tested for efficacy in selectively killing infected cells. Several immunotoxins have been found to be effective using infected in vitro cell lines. Initial studies using a murine retrovirus-induced neurodegenerative disease as a therapeutic model suggest that certain of the immunotoxins are also cytotoxic to infected cells in vivo. A retrovirus-induced lymphocytic leukemia is currently being evaluated as a second in vivo therapeutic model.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00418-09 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular biology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Maury	Staff Fellow	LPVD, NIAID
Other:	B. Chesebro	Chief	LPVD, NIAID
	W. Bushee	Summer Student	LPVD, NIAID

COOPERATING UNITS *(if any)*

Tom North	Dept. of Biochemistry	Univ. Montana

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The horse lentivirus, equine infectious anemia virus (EIAV), is a retrovirus closely related to the human immunodeficiency virus. EIAV is useful as a model for the study of retroviral expression and latency. In addition, a simple assay has been developed using EIAV infection to screen antiviral drugs.

A. The role of the Tat, or transactivator, protein in EIAV is being studied. We investigated the ability of EIAV Tat to upregulate EIAV gene expression in a number of different cell lines. Not all cell lines supported EIAV and HIV transactivation equally. All primate cell lines were unable to support EIAV transactivation, despite the ability of these cells to support high levels of HIV transactivation. Identification of the region(s) within EIAV and HIV Tat molecules responsible for the cell line specificity is currently ongoing.

B. Latency and viral reactivation in an EIAV-seropositive mare is being investigated. This mare has never been clinically ill with EIAV. No infectious virus has ever been detected in her. Upon corticosteroid treatment of the mare, viral sequences were detected in monocytes isolated from the mare ten days post treatment. *In vitro* differentiated macrophages derived from her monocytes were treated with mitogens. These treatments resulted in significant increases in the amount of detectable viral sequences detected by PCR amplification. The upregulation of viral expression by mitogens is currently being studied. In a related project, EIAV peptide specific antisera have been raised in rabbits. These antisera are currently being characterized and will be used to identify which EIAV proteins are being made in the EIAV sero-positive horse as well as other EIAV molecular constructs.

c. A viral infectivity assay has been developed that allows for relatively simple screening of potential antiviral drugs. This assay involves immunostaining of EIAV positive cells in the presence or absence of drugs in adherent cell lines. Since EIAV infects a number of different adherent cell lines from several different species, the role of the cell on the effect of antiviral drugs can be evaluated. The use of EIAV which is not infectious to humans to screen antivirals overcomes many of the biohazards involved in using HIV to identify new drugs. In collaboration with Dr. Tom North, EIAV reverse transcriptase is being purified from viral particles and drug interaction studies with the purified enzyme are under investigation.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00468-07 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Human AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Other:	W. Maury	Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
Irvin Chen, Department of Microbiology, UCLA School of Medicine, Los Angeles, CA; William O'Brien, Department of Microbiology, UCLA School of Medicine, Los Angeles, CA; Diane Griffin, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD.

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.4	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This project has continued to focus on studies of cell tropism of HIV infection. Our laboratory has developed a sensitive quantitative assay for HIV infection using CD4-positive HeLa cells. Most, but not all, HIV isolates infect these cells. Our recent experiments demonstrated that the block in infection by the nonpermissive isolates was due to lack of viral entry into the target cells. Using infectious molecular HIV clones of differing cell tropism recombinant HIV clones were synthesized, and it was found that a 120 base pair segment of V3 region of the viral envelope gene had a major influence on cell tropism. Interestingly, CD4-positive HeLa cells were infected at high efficiency by T lymphotropic virus strains, but were not infected by macrophage tropic strains. Thus, CD4-positive HeLa cells appear to be selective for T cell tropic HIV strains. Future studies are aimed at examining the cell tropism and V3 sequences of fresh HIV isolates from patients to determine the general relevance of these findings.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00524-05 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunologic Factors in Susceptibility and Resistance to Rabies Virus in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	D. Lodmell	Staff Scientist	LPVD, NIAID
	T. Ullrich	Staff Scientist	Ribi Laboratories, Hamilton, MT
	F. Hayes	Research Technician	LVP, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00550-04 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
EAE: Immunoregulation of Relapsing Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. L. Perry	Expert	LPVD, NIAID

Other: None

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00551-04 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Interactions During HIV Infection *In Vitro*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. L. Perry	Expert	LPVD, NIAID

Other: None

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00580-03 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemistry of Scrapie Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Caughey, Ph.D.	Senior Staff Fellow	LPVD
Other:	Richard Race, D.V.M.	Research Veterinarian	LPVD
	Bruce Chesebro, M.D.	Chief	LPVD
	John Coe, M.D.	Medical Officer	LPVD

COOPERATING UNITS *(if any)*
Drs. A. Dong and W. S. Caughey, Dept. of Biochemistry, Colorado State University, Ft. Collins, Colorado.

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.2	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical IIDEA project.

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The amyloidogenic, protease-resistant isoform (PrP-res) of the host protein PrP is specific to scrapie and related transmissible spongiform encephalopathies (TSEs) and its accumulation in the CNS is important in the pathogenesis of these neurodegenerative diseases. Our major goals are to determine 1) the mechanism of PrP-res formation in scrapie-infected cells, 2) methods of preventing PrP-res accumulation, and 3) the relationship of PrP-res to the transmissible scrapie agent.

Last year we reported studies of the kinetics and subcellular site of PrP-res formation in scrapie-infected neuroblastoma cells. Using this in vitro model, we have now identified potent inhibitors of the accumulation of PrP-res which are effective at concentrations as low as 1 ng/ml. These inhibitors include the amyloid-binding dye, Congo red, and several natural and synthetic sulfated glycan polyanions. Metabolic labeling studies indicated that Congo red has no effect on normal PrP metabolism and thus appears to selectively disrupt its conversion to PrP-res or destabilizes PrP-res once it is made. We have compared the inhibitory properties of a wide range of polyanions in an effort to define the structural requirements for good inhibitors of PrP-res accumulation. Such selective inhibitors should be valuable probes for studying the mechanism of PrP-res accumulation and the relationship of PrP-res to the transmissible scrapie agent. As an example of the latter application, we are now testing whether a decrease in the scrapie infectivity accompanies the reduction of PrP-res in Congo red-treated neuroblastoma cells. In vivo studies of the effects of Congo red on the development of scrapie in mice and hamsters are also underway. Because Congo red characteristically binds to all amyloids, including the ß-peptide fibrils of Alzheimer's disease, we speculate that it may be capable of inhibiting the accumulation of a broad spectrum of amyloidogenic proteins in vivo and have therapeutic value in treating amyloidoses.

Another approach we are taking to define the molecular mechanism of PrP-res formation is the comparison of the conformations of PrP-res and its normal isoform by infrared spectroscopy. The secondary structure analysis of PrP-res fibrils has been completed. We are continuing our refinement of both the infrared technique and a nondenaturing immunoaffinity purification of normal PrP so that we can complete the comparison.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00610-02 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of H-2 I-E in recovery from Friend virus induced leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. L. Perry	Expert	LPVD, NIAID
Other:	M. Miyazawa	Visiting Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00611-02 LPVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enrichment of Hematopoietic Stem Cells from Mouse Bone Marrow

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.J. Spangrude	Senior Staff Fellow	LPVD, NIAID

Other:

COOPERATING UNITS *(if any)*
Metabolism Branch, National Cancer Institute, NIH (L. Staudt)

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The goal of this project is to identify control mechanisms in the early stages of hematopoiesis in a mouse model system, utilizing methodology for obtaining highly enriched populations of hematopoietic stem cells (HSC) from normal mouse bone marrow tissue. By isolating these cells to separate them from the majority of more mature bone marrow cells, one can ask a variety of questions regarding the nature of HSC in the mouse. These include the identification of surface antigens expressed HSC, the functional heterogeneity of the stem cell compartment at a single cell level, and the susceptibility of HSC populations to infection by pathogenic mouse viruses and by retroviral constructs. Such viruses may be exploited as vectors to introduce new genetic material into the stem cell compartment. Further, in vitro culture systems and growth factors are being tested for the ability to propagate and induce self-renewal of the enriched HSC populations.

Pursuant to the goals of this project, it is critical to develop confidence in the methodology utilized to enrich the HSC population. In particular, the enrichment method should select for all primitive HSC present in the bone marrow, and not simply a subpopulation which may or may not include the most primitive members of the HSC compartment. Our results demonstrate significant variation of HSC phenotype among different mouse strains. These variations include variable expression of two major cell surface markers of HSC in mice, Ly-6A/E and Thy-1. Fortunately, one mouse strain has been identified in which the vast majority of HSC activity can be identified and isolated by immunofluorescent staining and flow cytometry.

Separation of HSC by staining with a fluorescent mitochondrial dye has allowed identification of resting and activated subsets of HSC. These isolated subsets are very similar functionally, but differ in self-renewal potential. As a result, these HSC subsets will be useful in defining in vitro assays that are capable of detecting HSC self-renewal. This will result in assays for HSC that can be applied to human tissues and which will eventually reduce the requirement for experimental animals in HSC research.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

LABORATORY OF VIRAL DISEASES
1992 ANNUAL REPORT
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LABORATORY OF VIRAL DISEASES
ANNUAL REPORT, 1992
SUMMARY

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. These studies are designed to increase fundamental knowledge as well as to facilitate the development of new approaches to the prevention and treatment of virus infections. Current topics of basic research include virus entry into cells, regulation of gene expression, mechanisms of DNA replication, assembly and transport of viral proteins and particles, action of virus growth factors, determinants of virus virulence, host resistance genes, and viral targets of humoral and cellular immunity. Applied areas of investigation include the development of recombinant expression vectors, candidate vaccines, and antiviral agents. These studies involve a wide range of DNA and RNA viruses including human immunodeficiency virus.

REGULATION OF VIRUS GENE EXPRESSION

Viruses provide advantageous systems for studying basic aspects of gene expression and can be used as expression vectors. Several different virus systems are currently under investigation by members of LVD.

POXVIRUSES. Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. In order to express and replicate their genomes outside of the nucleus, poxviruses must encode enzymes for these processes. This feature make poxviruses, among which vaccinia virus is the prototype, unique for studying the regulation of transcription. Additional information regarding transcription also is needed to improve vaccinia virus expression vectors, which are currently being evaluated as live vaccines against several diseases including AIDS.

Identification of two additional RNA polymerase core subunits. The DNA-dependent RNA polymerase that is packaged in infectious vaccinia virus particles is virus-encoded. During the past year, two additional RNA polymerase subunit genes, *rpo18* and *rpo7*, encoding polypeptides of 18,000 and 7,000 Daltons, were located within the vaccinia virus genome. The 63 amino acid *rpo7* polypeptide appears to be homologous to the smallest subunit of eukaryotic RNA polymerases and a metal binding motif, Cys-X-X-Cys-Gly, is located at precisely the same location near the N-terminals of the two proteins. By contrast, the sequence of *rpo18* does not resemble that of any known eukaryotic or prokaryotic gene. Two forms of the *rpo18* gene product with apparent Mr of 21,000 and 22,000 were present in the viral RNA polymerase. Subunits encoded by 8 different viral genes are now known to be associated with the core RNA polymerase.

(Ahn, Amegadzie, and Moss)

RNA polymerase-associated transcription specificity factor. A transcriptionally active form of vaccinia virus RNA polymerase was separated from the core polymerase by column chromatography. The active form contained an additional polypeptide with an apparent Mr of 85,000. Peptide mapping demonstrated that the polypeptide is encoded by the H4 gene of vaccinia virus which is predicted to encode a protein of 94,000 Daltons. Because of its association with RNA polymerase, the gene was named *rap94*. The role of the protein in mediating specific transcription was demonstrated using an extract from cells in which the *rap94* gene was transiently expressed.

(Ahn and Moss)

Mutational analysis of the core, spacer, and initiator regions of vaccinia virus intermediate-class promoters. Activation of vaccinia virus late gene transcription is dependent on DNA replication and the expression of three genes: A1L, A2L, and G8R. Deletional mutagenesis demonstrated that the regulatory elements of all three promoters extended between 20 and 30 nucleotides upstream of their RNA start sites. Single-base substitutions of the G8R promoter revealed two critical elements located from -26 to -13 (the core element) and -1 to +3 (the initiator element). Additional mutations defined the TAAA sequence as the critical initiator element. The length, but not the nucleotide sequence, of the segment between the core and initiator regions was crucial. The requirement for the spacer to be 10 or 11 nucleotides was consistent with a single turn of a double helix. The A1L and A2L promoters resembled the G8R promoter, and mutations in the conserved bases had the predicted effects on expression. We concluded that the three intermediate promoters are composed of a 14-bp A+T-rich core sequence separated by one turn of the double helix from the TAAA initiator element.

(Baldick, Keck and Moss)

Poly(A) polymerase and a dissociable polyadenylation stimulatory factor encoded by vaccinia virus. mRNA made in eukaryotic cells typically has a 3′ poly(A) tail that is added postranscriptionally. To investigate the mechanisms by which 3′ poly(A) is formed, we identified the genes for the two vaccinia virus-encoded polypeptides, VP55 and VP39, which comprise the heterodimeric poly(A) polymerase. Free VP55, obtained first by antibody mediated dissociation of the heterodimer and subsequently by recombinant DNA methods, possessed catalytic polyadenylation activity whereas free VP39 did not. VP55 added 30 to 35 adenylates in a rapid, processive burst to a primer representing the 3′ end of a viral mRNA after which polyadenylation decelerated dramatically and became non-processive. VP39 greatly stimulated polyadenylation and allowed the formation of long poly(A) tails.

(Gershon and Moss)

Cap-specific mRNA (nucleotide-$O^{2'}$-)-methyltransferase and poly(A) polymerase stimulatory activities of vaccinia are mediated by a single protein. The vaccinia virus gene for S-adenyosyl-L-methionine:mRNA (nucleotide-$O^{2'}$-)-methyltransferase, an enzyme required for the formation of the 5' cap structure of mRNA, was identified. Protein sequence analysis revealed that this cap-specific methyltransferase is derived from the same open reading frame as that previously shown to encode VP39, a M_r 39,000 dissociable subunit of poly(A) polymerase that stimulates the formation of long poly(A) tails. Consistent with this finding, methyltransferase activity was associated with the heterodimeric poly(A) polymerase, which is composed of VP55 and VP39 subunits, as well as with monomeric VP39 protein isolated from vaccinia virions or produced by recombinant DNA methods. From these data, we concluded that the same protein functions as a methyltransferase and a poly(A) polymerase stimulating factor to modify the 5' and 3' ends of mRNA, respectively.

(Schnierk, Gershon and Moss)

PARVOVIRUSES. Parvoviruses are small viruses that contains a single-stranded DNA genome. One well studied member of his family, adeno-associated virus (AAV), is dependent for replication on coinfection with an adenovirus or herpesvirus. AAV gene expression appears to depend on cellular and helper viral gene products and is regulated by post-transcriptional mechanisms that also may be important for eukaryotes.

Regulation of synthesis of AAV structure proteins at the translational level. Infectious AAV plasmids containing site-directed mutations were used to confirm that structural protein B is initiated at ACG, a previously unknown eukaryotic translation initiation codon. Furthermore, mutations of this codon affected the levels of structural protein B as well as that of structural protein C, both of which are expressed from the same transcript. Thus, the coordinate expression of B and C depends on the relatively weak ACG initiation codon of B. The initiation codon for the third structural protein A (an AUG) was also definitively mapped using this same technique.

Sebring, Muralidhar and Rose)

Regulation of synthesis of AAV structural proteins by alternative splicing. The only functional mRNA for expression of structural protein A is generated by alternative splicing. A mutation in the splice acceptor site of the B/C message abolishes the synthesis of B and C while allowing synthesis of A. The splice acceptor site of the A message has been specifically located and, when mutated, abolishes the synthesis of only A.

(Sebring, Muralidhar and Rose)

VIRUS DNA REPLICATION

Viruses are useful systems for analyzing the diversity of mechanisms employed in DNA replication. In addition, the virus encoded factors provide potential targets for chemotherapy.

HERPES SIMPLEX VIRUS (HSV). HSV and other members of the herpesvirus family are significant human pathogens. The study of HSV DNA synthesis is useful as a model for eukaryotic DNA replication and for designing new anti-viral strategies. Previous work from this laboratory demonstrated that seven HSV genes are necessary and sufficient for authentic origin-dependent DNA replication. Current efforts are directed toward studying this process with purified proteins.

Studies on the polymerase processivity subunit, UL42. The HSV-1 DNA polymerase consists of a heterodimer of the UL30 (Pol; the catalytic subunit) and UL42 polypeptides. Our previous data has shown that the role of UL42 in the DNA polymerase complex is to act as a "clamp", decreasing the probability that the polymerase will dissociate from the template after each cycle of catalysis. Studies of mutated derivatives of UL42 have shown that the carboxyl-terminal third of the polymerase accessory factor, UL42, is not essential for the function of the protein in DNA replication, and a nuclear localization signal for this polypeptide is located near its amino terminus.

(Gottlieb, Lantz, Franco and Challberg)

Mutational analysis of the UL52 component of the HSV-1 helicase-primase complex. The UL52 gene product of HSV-1 comprises one subunit of a 3 protein helicase-primase complex essential for replication of viral DNA. On the basis of amino acid sequence motifs, UL5 may contain the helicase active site and, since UL8 is required for neither activity, it has been suggested that UL52 may be the primase. Base substitution mutations were introduced into 5 regions of the UL52 gene that are highly conserved among HSV and the related herpes viruses HCMV, EBV and VZV. Of 7 mutants analyzed by an *in vivo* complementation assay, 3 mutants, in 3 different conserved regions of the protein, failed to support DNA replication.

(Klinedinst, Sherman, and Challberg)

Characterization of the origin binding protein. We have previously shown that the protein encoded by the HSV UL9 gene has two functions characteristic of replication initiator proteins: it binds specifically to the origins of replication, and it has an intrinsic DNA binding protein, ICP8, and direct measurements have verified the existence of an interaction between the two proteins. A truncated derivative of UL9 comprising the carboxyl-terminal 317 amino acids retains the origin binding activity of the full length protein, but exists as a monomer rather than a dimer in solution and fails to link the two binding sites at the origin by protein-protein interaction.

(Fierer and Challberg)

POXVIRUSES. Poxviruses provide a unique experimental system, since the enzymes and factors are encoded within the virus genome and replication occurs in the cytoplasm. Previous studies revealed that vaccinia virus DNA is replicated as concatemeric structures which are resolved into unit length genomes with hairpin ends.

Mutagenesis of a conserved region which may contain the nucleotide binding site of the vaccinia virus DNA polymerase. Following marker transfer with a PCR product synthesized with a priming oligonucleotide containing linked alterations, aphidicolin resistant virus was selected and viral DNA was shown to contain a codon for tryptophan which had replaced one for tyrosine. This and other results show that a vaccinia virus plaque assay can provide an *in vivo* test for mutations that may affect the function of an important site in a class of DNA polymerase.

(DeFilippes)

Identification and characterization of the vaccinia virus gene encoding the nicking-joining enzyme. A nuclease has been detected in virions which converts plasmids containing the vaccinia virus concatemer junction into nicked circular, linear, and cross-linked linear molecules by a region-specific cleavage near the border of the hairpin loop. We have isolated the predominant protein that chromatographs with these activities and identified the product as corresponding to open reading frame A5L, a late gene of approximately 33 kd. Current efforts are directed towards characterization of the protein product and its role in DNA processing and viral growth.

(Levy and Merchlinsky)

Identification of other proteins involved in telomere resolution. A search of the vaccinia virus Copenhagen strain viral genome by Ken Abremski using an algorithim he developed to detect members of the integrase family of site-specific recombination proteins detected one open reading frame in vaccinia, G9R, as the sole candidate for an integrase. The protein encoded by this open reading frame has an unknown function, has a molecular weight of approximately 39kd, and the DNA has the characteristic nucleotide sequence of the late class of viral genes. Attempts to demonstrate that this gene product was the only component required for telomere resolution were unsuccessful. Current efforts are designed to determine if G9R participates in telomere resolution by purification and characterization of the protein and the design of viral genomes that conditionally express G9R to determine what effect its absence has on DNA processing.

(Merchlinsky)

ADENO-ASSOCIATED VIRUS. The AAV genome is a linear single-stranded DNA molecule with partially palindromic inverted terminal repeats. Replication of AAV DNA occurs by a mechanism involving self-primed synthesis.

Synthesis of concatemeric AAV DNA without AAV-specified proteins. Following coinfection of restrictive monkey cells with AAV2 and human adenovirus 5, NS and structural AAV protein synthesis was inhibited > 98% and AAV DNA synthesis occurred but instead of normal unit length duplex and single stranded molecules, long concatemers accumulated. This restriction of AAV replication was completely reversed by preinfection with SV40 or by using a simian adenovirus as a helper. Taken together, these results suggest that synthesis of AAV concatemeric DNA may not require AAV nonstructural proteins, but that one or more of these proteins is required to resolve AAV concatemeric intermediates to unit length duplex DNA molecules, presumably by nicking at a specific site(s).

(Sebring and Rose)

HSV genes enhance AAV DNA synthesis. AAV DNA synthesis was efficiently induced by contransfections of vero cells with and infectious AAV plasmid (pAV1) and clones at least five of the seven HSV-required genes plus ICP4. In contrast to their requirement for HSV DNA synthesis, none of the five HSV-required genes was absolutely necessary for AAV DNA replication. Furthermore, they appeared to exert a cumulative effect on the stimulation of AAV DNA synthesis. This suggests that products of these genes may be enhancing AAV DNA synthesis by participating in a DNA replication complex that is partially composed of cellular DNA synthesizing factors.

(Mishra and Rose)

Information regarding the folding, assembly, and transport of viral glycoproteins and the formation and budding of virus particles is of intrinsic interest and practical importance, since it determines the antigenic structure recognized by neutralizing antibody.

HUMAN IMMUNODEFICIENCY VIRUS (HIV). HIV is the etiological agent of acquired immune deficiency syndrome (AIDS). At present, there is not effective vaccine against this virus and therapeutic agents provide only limited help. The envelope protein is the best target for neutralizing antibody and is therefore of particular importance for development of a vaccine.

Regions of CD4 and HIV-1 env involved in membrane fusion. HIV-1 infection of human T-cells and macrophages is initiated by binding of the external subunit (gp120) of the viral envelope glycoprotein (env) to CD4 molecules on the target cell surface. Direct fusion then occurs between the virion and cell membranes by a poorly understood process which is believed to involve CD4-induced structural changes which activate env for fusion. Specific regions of both CD4 and gp120 have been implicated in fusion, distinct from any involvement in binding. We employed site-directed mutagenesis to probe the roles of these regions in membrane fusion and CD4-induced structural changes in env (gp120 release). Our results challenge the notion of a critical role of the so-called CDR3 region of CD4 in membrane fusion and gp120 release. We have confirmed the requirement of specific sequences in the V3 region of gp120 for fusion, but not for gp120 release.

(Broder, Sisler, Earl, and Berger)

Additional molecular components critical for CD4/env-mediated fusion. Membrane fusion mediated by CD4/env generally requires that the human CD4 molecule be expressed on a human cell type. We observed that the block to fusion in animal cells expressing human CD4 cannot be overcome by overexpressing CD4 at extremely high levels. Using a video fluorescence microscopy dye transfer assay, we observed that probe cells expressing env readily fuse with animal/human hybrid giant cells expressing CD4, independent of whether the CD4 is originally expressed on the animal or human cell. These results argue against the presence of a dominant inhibitory component on the animal cell; rather, they suggest that human cells contain an accessory component(s) required for fusion. Preliminary results indicate that the human component can be functionally transferred by fusing human plasma membrane vesicles to CD4-expressing animal cells. We have also found that fusion is strictly dependent on calcium ions.

(Broder, Nussbaum, and Berger)

Human and simian immunodeficiency virus envelope dimers bind multiple CD4 molecules. The envelope (env) glycoproteins of human and simian immunodeficiency virus (HIV and SIV) form non-covalently associated oligomers which mediate virus binding to the cell surface and fusion between the viral envelope and plasma membrane. Several approaches were used to determine whether the env protein oligomers exhibit multimeric CD4 binding. The assays were based on the use of two different length truncated CD4 molecules, only one of which could bind to OKT4 monoclonal antibody. We reasoned that OKT4 would immunoprecipitate the shorter CD4 molecule if it were linked to the same env oligomer as the longer one. Several variations of this approach all demonstrated multimeric CD4 binding by env oligomers but not env monomers. Multimeric CD4 binding may have important implications for env-protein-CD4 avidity, CD4-induced release of gp120, subunit-subunit cooperativity during virus membrane fusion, as well as therapeutic strategies.

(Earl, Doms and Moss)

Overexpression of the HIV-1 Gag-Pol polyprotein results in intracellular activation of HIV-1 protease and inhibition of assembly and budding of virus-like particles. The consequences of altering the ratios of the Gag and Pol proteins were tested using vaccinia virus expression vectors, in which the gag and pol genes were fused by placing them in the same open reading frame. Large numbers of budding, immature, and mature retrovirus-like particles were visualized by electron microscopy when the wild type gag-pol gene was expressed, whereas no particles were detected in cells that expressed the fused gag-pol gene. The block to virus assembly was partially overcome by (i) inhibition of the HIV-1 protease with a peptidomimetic inhibitor, (ii) mutagenesis of the active site of the protease, or (iii) shortening of the Gag-Pol polyprotein by deletion of most of the reverse transcriptase gene. Nevertheless, budding was inefficient and the particles appeared immature and frequently aberrant. These results indicated that overproduction of the full-length Gag-Pol polyprotein and increased intracellular protease activity were both detrimental to viral assembly. Further experiments indicated that intracellular processing of Gag and Gag-Pol polyproteins occurred in the absence of particle formation when myristoylation was prevented.

(Karacostas, Wolffe and Moss)

INFLUENZA VIRUS. The trimeric influenza hemagglutinin (HA) molecule is the best characterized viral envelope protein and therefore serves as an excellent model. Additionally, influenza virus remains a significant human pathogen, and the HA is a critical antigen for protecting individuals against disease.

Folding, assembly, and transport of viral glycoproteins. An antibody that binds a cryptic antigenic site on the influenza virus hemagglutinin was used to select a unique set of virus mutants. These mutants possessed single amino acid substitutions located a considerable distance from the antibody binding site. The amino acid substitutions diminish antibody binding by decreasing the conformational flexibility of the portion of the HA containing the antibody binding site. This represent a novel mechanism for a virus to escape antibody mediated neutralization.

(Yewdell and Bennink)

VACCINIA VIRUS. There are two types of infectious vaccinia virus particles: intracellular naked virions (INV) and extracellular enveloped virions (EEV). The latter form of the virus is thought to be important for virus spread and virulence.

Vaccinia virus morphogenesis is interrupted when expression of the gene encoding an 11-kilodalton phosphorylated protein is prevented by the escherichia coli lac repressor. A conditional lethal vaccinia virus mutant, which constitutively express the Escherichia coli lac repressor and has the lac operator controlling the vaccinia F18R gene encoding an 11-kDa protein, was previously shown to be dependent on the inducer isopropyl-β-D-thiogalactoside (IPTG) for replication. Pulse-chase experiments revealed that proteolytic processing of the major viral structural proteins, P4a and P4b, was inhibited under nonpermissive conditions, suggesting a block in virus maturation. Without addition of IPTG, the failure of virus particle formation was indicated by sucrose gradient centrifugation of infected cell lysates and by the absence of vaccinia virus-mediated pH-dependent cell fusion. Electron microscopic examination of infected cells revealed that immature virus particles, with aberrant internal structures, accumulated when synthesis of the 11-kDa DNA-binding protein was prevented.

(Zhang and Moss)

Immature viral envelope formation is interrupted at the same stage by lac operator-mediated repression of the vaccinia virus D13L gene ond by the drug rifampicin. We constructed a recombinant vaccinia virus in which elements of the Escherichia coli lac operator system were used to regulate the D13L gene. Replication of the recombinant vaccinia virus was dependent on addition of the inducer IPTG and the virus yield was decreased by more than 99% when IPTG was omitted. Under the nonpermissive condition, transcription of the D13L gene was reduced and synthesis of the 65,000-Da protein product was inhibited by more than 95%. Consequently, virion morphogenesis was blocked at an early stage and uncoated membrane precursors of the immature viral envelope and uncleaved precursors of the major core proteins accumulated. The phenotype of the conditional lethal mutant virus, in the absence of IPTG, closely resembled that of wild-type virus in cells treated with rifampicin.

(Zhang and Moss)

Role of cell-associated enveloped vaccinia virus in cell-to-cell spread. The roles of intracellular naked (INV), cell-associated enveloped (CEV), and extracellular enveloped (EEV) forms of vaccinia virus in cell-to-cell and longer range spread were investigated by using two closely related strains of vaccinia virus, WR and IHD-J. WR and IHD-J produced similar amounts of INV and formed similar-size primary plaques but IHD-J produced 10 to 40 times more EEV and spread to distant cells much more efficiently than did WR. Nevertheless, cells infected with WR and IHD-J had similar amounts of CEV, indicating that wrapping and transport of WR virions were unimpaired. The low amount of WR EEV could be attributed to retention of CEV on the cell membrane. In support of this hypothesis, mild treatment with trypsin released as much or more infectious virus from cells infected with WR as it did with cells infected with IHD-J. Most of the virus released by trypsin sedimented with the buoyant density of EEV. Also, addition of trypsin to cells following inoculation with WR led to comet-shaped distribution of secondary plaques characteristic of IHD-J. These results demonstrated that the release of CEV from the cell surface was limiting for extracellular virus formation and affirmed the role of EEV in a long-range spread.

(Blasco and Moss)

CELL BIOLOGY

Confocal microscopy of cells. The laser confocal scanning microscope (LCSM) provides unparalleled resolution for localizing fluorescent probes in cells. The LCSM is being used to determine the subcellular distribution of proteins, to follow the intracellular trafficking of newly synthesized membrane proteins, and to study the migration of developing thymocytes in the thymic explants.

(Yewdell and Bennink)

Mechanism of brefeldin A action. Brefeldin A is a fungal metabolite with the unique ability to interfere with vesicular trafficking. Two fluorescent analogs were prepared that maintain biological activity. Fluorescent microscopy revealed that the BFA analogs localized primarily to the endoplasmic reticulum of live or aldehyde fixed cells, suggesting that BFA function might in part be related to perturbations in the ER membrane. These conjugates should be of use as markers for localizing the endoplasmic reticulum in fixed cells and as probes of endoplasmic reticulum structure in living cells.

(Deng, Yewdell and Bennink)

VIRAL IMMUNOLOGY

Class I molecules of the major histocompatibility complex class (MHC) consist of a highly polymorphic heavy chain complexed to B_2-macroglobulin. Class I molecules are expressed on virtually all cell types. Their sole function is to bind antigens and present them to T cell bearing CD8 molecules. These T cells are known as cytotoxic T lymphocytes (CTLs) due to their ability to lyse histocompatible cells in an antigen specific manner. CTLs play a critical role in eradicating intracellular pathogens and tumors. On the negative side, they are involved in organ rejection, and in many autoimmune dyscrasia.

Proteolytic generation of antigenic peptides. To examine where and how cells produce the antigenic peptides recognized by T lymphocytes in a complex with class I molecules of the major histocompatibility complex, we produced a number of new recombinant vaccinia viruses. One expresses a form of the cell protein ubiquitin altered to interfere with ubiquitin-targeted proteolysis. Another expresses a cytosolic form of a class I molecule designed to probe the cytosol for the presence of antigenic peptides.

(Cox, Bacik, Yewdell and Bennink)

Structure and function of peptide transporters. It was recently discovered that the major histocompatibility complex (MHC) encodes two proteins that seem to specifically transport antigenic peptides from the cytosol to the secretory compartment where the peptides bind to other MHC encoded molecules that carry the peptides to the cell surface for immune recognition. To help characterize the transporters, their genes were inserted into vaccinia virus. The resulting recombinants are being used mainly to characterize the intracellular location and function of the proteins.

(Esquivel, Bacik, Eisenlohr, Anderson, Bennink and Yewdell)

Delivery of antigens to the MHC class I processing pathway. Methods were explored for delivering protein antigens to cells that enable the cells to properly process the antigens for recognition by major histocompatibility complex (MHC) class-I restricted T lymphocytes. Conjugation of a model antigen (ovalbumin) to folic acid enhanced its presentation to T cells. Presentation of folate-conjugated proteins is being characterized to determine if this is due to increased delivery to the cytosol, where antigen processing is thought to occur.

(Anderson, Bennink and Yewdell)

Class I molecule assembly and trafficking. Class I molecules of the major histocompatibility complex (MHC) bind peptide antigens and carry them to the cell surface for recognition by T lymphocytes. Antigenic peptides were recovered from class I molecules present in an early secretory compartment in cells treated with the fungal metabolite, brefeldin A, demonstrating that peptide association occurs early in the transport of newly synthesized class I molecules to the cell surface. To provide finer resolution of the antigen association compartment in future studies, a recombinant vaccinia virus was produced that expresses a class I molecule retained in the endoplasmic reticulum.

(Esquivel, Day, Lapham, Deng, Bennick and Yewdell)

Antigen presentation by human tumors. The failure of the immune system to prevent malignancies may be due to the selection of mutant tumor cells unable to effectively present tumor antigens for recognition and lysis by T lymphocytes. A method has been developed to rapidly measure the ability of tumor cells to present antigens for cytotoxic T lymphocyte recognition. Using this method, it was found that a high percentage of human tumor cells were unable to present antigens to T cells. In some cases, this could be attributed to down-regulation of antigen processing machinery encoded by the major histocompatibility complex (MHC). In others, cells selectively failed to express β_2 microglobulin, a critical component of the structure recognized by T cells.

(Esquivel, Bennink, Yewdell)

VIRUS PATHOGENESIS

Recent advances in recombinant DNA technology, cell biology and immunology have provided an unparalleled opportunity to study the fine detail of the virus and host genes involved in virus pathogenesis. The study of vaccinia virus (VV) and cowpox virus (CPV) in the rabbit or chick embryo, and especially ectromelia virus (EV) in the mouse have a number of advantages over other virus/animal models. For example with ectromelia virus infections of the mouse: 1) there are disease-resistant and -susceptible strains of mice available; 2) the natural route of infection is known and can be mimicked by the foot-pad route; 3) the natural history of the disease (mousepox) is well understood; and 4) experimental virus transmission between an index mouse and contacts is reproducibly attainable in the laboratory.

POXVIRUS-ENCODED VIRULENCE GENES

Vaccinia virus complement control protein (VCP). VCP, a secreted protein produced by the C2IL gene of VV, was shown to block the enhancement of antibody-dependent neutralization of VV induced by the alternative pathway of complement activation. This result, together with our previous data, demonstrates that VCP inhibits both pathways of complement activation. A mutant of VV with deleted C2IL gene produced smaller skin lesions than wild-type VV in rabbits as well as guinea pigs that are deficient in the classical pathway of complement activation. Thus, VCP appears to play an important role in the resistance of poxviruses to humoral immune mechanisms.

(Isaacs and Moss)

Interferon-resistance of vaccinia virus. The anti-viral effect of interferon is mediated in part by the phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF-2α) by the double-stranded RNA activated (DAI) protein kinase. Vaccinia virus contains a gene, K3L, that encodes a homolog of eIF-2α. We have shown that the K3L protein inhibits eIF-2α phosphorylation and DAI kinase activation.

(Moss)

Cowpox anti-inflammatory protein. The 38-kDa protein inhibits the inflammatory response in the chorioallantoic membrane (CAM) of the chick embryo. Others have shown this protein to affect the maturation of Il-1 β by inhibiting the action of the Il-1 β convertase, and we have found the protein to interfere with the generation of a 14,15-diHETE molecule form the lipoxygenase pathway of arachidonic acid metabolism. During the past year, we have attempted to show that the 14, 15-diHETE is a chemoattractant for the human neutrophil, but with no success. Addition of this compound to the CAM of the chick embryo also failed to demonstrate chemotactic activity, however chemotaxis could be shown if a second signal was provided. Thus, it is still not clear if the 14, 15-diHETE is directly or indirectly involved in the chemotactic signal generated in the CPV-BR 38kDa minus infection of the chick embryo CAM or just a correlate.

(Palumbo and Buller)

Ectromelia virus tissue tropisms. The host range of EV in tissue culture was examined with a battery of 12 cell lines from six animal species, and EV replication was found to be more restrictive than two other orthopoxviruses (CPV and VV). The EV CHO gene was found to be non-functional, and the EV K1L and C7L genes only partially active, as the EV K1L supported replication in Hep-2 cells, but not RK13 or SIRC cells and the C7L gene permitted growth in MRC-5 but not Hep-2 cells. DNA sequence analysis of the EV CHO gene found 12 deletions, 4 insertions and 72 substitutions over the course of the 1822 nucleotide sequence relative to the CPV CHO gene sequence. As compared to the VV K1L sequence, the EV K1L homologue showed only seven conservative changes and the deletion of an A at nucleotide 932 which resulted in a change of a Lys to an Arg at amino acid 282, and the insertion of a Thr at amino acid 284, 1 residue from the carboxy terminus. The DNA sequence of C7L differed from its' VV counterpart in having four conservative changes and two nonconservative changes. A knockout mutant of the EV K1L gene showed a pathogenesis picture in the mouse no different from the EV WT. These data suggest that an intact EV CHO and EV K1L gene are not required for maintenance of EV in nature.

(Chen and Buller)

An in vivo ectromelia virus replication mutant. An EV ORF predicted to encode a p28 protein was sequenced and found to contain a zinc finger motif that is found in a select group of proteins some of which have transcription functions. An EV lacking this ORF was shown to replicate as efficiently as WT in tissue culture, but replicated at least a 100-fold less efficiently than WT EV in the foot-pad of the mouse, spread more slowly from the primary site of inoculation to internal organs and had an LD_{50} that was 1,000 times higher than the WT virus. The p28 protein has been over expressed and will be tested for DNA binding activity.

(Senkevich and Buller)

Ectromelia virus replication and spread in C57BL/6 mice. Ectromelia virus infection of the C57BL/6 mouse results in the detection of virus infectivity first in the popliteal lymph node draining the site of infection and subsequently in the spleen and liver. Virus infectivity levels dramatically decrease in the spleen and liver at 7 days p.i., presumably due to development of cell-mediated immunity especially CTL. Virus infectivity persists as late as 12 days p.i. in the primary site of infection as well as the popliteal lymph node. Examinations of the popliteal lymph node through electron microscopy, light level histology and in situ hybridization only detected virus replication transiently up to day 4. This suggests that the popliteal lymph node is refractile to virus infection during the later times of the disease, and the virus infectivity that is observed is simply virus carried to the lymph node from the primary site of infection. This study suggests that evaluation of the contribution of various virus-encoded genes on virus pathogenesis will be best achieved by studying the epidermis and dermal regions of the primary site of infection.

(Karupiah and Buller)

Molluscum contagiosum virus replication in an animal model. Approximately thirteen percent of AIDS patients have molluscum contagiosum virus (MCV) lesions. These lesions are frequently found on the face, and are larger and more numerous than observed with HIV-1 seronegative individuals. The current treatment regimens are far from optimal, and the development of new therapies has been hampered by the in ability to grow the virus either in vitro or in an animal model. The purpose of this project is to develop a method for propagating the virus in the laboratory which will allow a more detailed study of its' replication cycle and provide a means to study the effects of promising anti-viral compounds. After trying a number of unsuccessful approaches, we have obtained MCV replication in approximately 25% of human foreskin grafts to nude mice. Of interests was the incubation period of greater than 128 days to observe gross pathology.

(Buller)

Use of expression vectors has become an important part of recombinant DNA technology. Vaccinia virus is now widely used for expression of proteins in mammalian cells and has proven particularly useful for determining the targets of humoral and cellular immunity. Recombinant vaccinia virus is currently being tested as a candidate AIDS vaccine in humans and a rabies vaccine in wild-life. The unique DNA integration and other properties of some parvoviruses suggest that they might be of particular use for gene therapy (see section on antiviral agents).

Molecular construction of chimeric vaccinia virus genomes by ligation of DNA: recombination independent production of viral genomes. A new recombination independent method for production of viral genomes containing insert DNA which relies on ligation of the insert DNA to subgenomic pieces of the vaccinia DNA genome, the transfection of the ligation mix into cells infected with a conditionally defective virus, and the isolation of the genomes containing inserts by plaque assay was developed. Vaccinia virus vectors were designed with a site for the infrequent cutting restriction enzyme NotI positioned so that colorometric detection or resistance to bromodeoxyuridine could be used to identify genomes containing insert DNA. This protocol generates containing insert DNA more than one hundred times as efficiently as by recombination and was independent of size of the insert up to 26,000 base pairs.

(Merchlinsky and Moss)

SP6 RNA polymerase containing vaccinia virus for rapid expression of cloned genes in tissue culture. A hybrid transient expression system in which tissue culture cells are infected with a recombinant vaccinia virus encoding bacteriophage T7 DNA-dependent RNA polymerase and transfected with a plasmid containing a cloned gene behind the bacteriophage T7 promoter allows rapid high level expression in nearly 100% of the cells. In order to extend this system to clones from libraries containing SP6 promoters, a new vaccinia virus was constructed encoding bacteriophage SP6 RNA polymerase. Expression obtained with this system was equivalent to that achieved with the bacteriophage T7 RNA polymerase.

(Isaacs and Moss)

Non-replicating, safety-tested vaccinia vector efficiently expresses recombinant genes. The eradication of smallpox was achieved though immunization with live vaccinia virus. Presently, vaccinia is used extensively as a gene expression vector and is under evaluation as a recombinant vaccine. Because vaccinia is infectious, its use in the laboratory has been hindered by safety concerns and regulations. For general vector applications, these problems might be alleviated by the adoption of a highly attenuated vaccinia strain such as the modified virus Ankara (MVA), which is avirulent in immunosuppressed animals and was safely used for primary vaccination in over 120,000 humans including high risk patients. MVA cannot replicate in human and most other mammalian cells tested, however, and high expression of recombinant genes seemed unlikely in view of reports that other host range vaccinia mutants are inhibited early in infection. Surprisingly, late viral and recombinant gene expression is unimpaired in non-permissive human cells, and we have demonstrated that MVA can be used to produce highly efficient and exceptionally safe vectors.

(Sutter and Moss)

New molecular approaches are being developed for the prevention and treatment of viral diseases. These include recombinant subunit and live vaccines, targeted therapeutics, and novel ways of providing intracellular immunity.

CD4-Pseudomonas exotoxin - candidate HIV therapeutic. A genetically engineered hybrid toxin, CD4-PE40, composed of a region of CD4 containing the HIV envelope glycoprotein binding site linked to active domains of *Pseudomonas* exotoxin A, was produced as a collaborative project between NIAID and NCI. CD4-PE40 has been licensed by the Upjohn pharmaceutical company and Phase I clinical trials were initiated 8/91 at NIH. A multicenter expanded Phase I trial is planned by the AIDS Clinical Trials Group for this year. Support for the clinical trials was obtained by recent in vitro findings that CD4-PE40 potently inhibits primary HIV-1 isolates known to be highly refractory to neutralization by soluble CD4. These results have important conceptual and practical implications for CD4 based therapeutics.

(Kennedy, Moss and Berger)

Intracellular antibody-mediated virus neutralization. An antibody specific for an internal influenza virus structural protein was identified that specifically blocks influenza virus infection if the antibody is introduced into the cytosol by electroporation. This antibody is able to block infection with a broad range of influenza A viruses. Genes encoding the heavy and light chains of this antibody were cloned, and the portions encoding the ER insertion sequences were removed. These modified genes were used alone or in combination to transfect cells. These cells being characterized for their resistance to influenza virus infection, with the goal of producing transgenic animals resistance to infection with influenza A virus.

(FFrench, Yewdell and Bennink)

Parvovirus (AAV) vectors provide intracellular resistance to HIV-1. Stable resistance to HIV-1 was induced in human lymphocyte cell lines using a recombinant AAV-based vector encoding an antisense RNA complementary to the HIV-1 TAR sequence, which is common to all HIV transcripts and critical for efficient virus transcription and replication. Cell clones that constitutively expressed this antisense RNA, specifically inhibited (>95%) HIV LTR-directed gene expression, HIV replication, and production of infectious HIV particles.

(Chatterjee, Wong, Rose and Johnson)

Parvovirus (AAV) vectors provide intracellular resistance to herpes simplex virus (HSV). An AAV-based antisense vector targeting the early HSV-encoded transactivator, ICP4, was used to transduce stable intracellular resistance to HSV-1 as well as HSV-2. A 1,000- to 10,000-fold inhibition of HSV production, correlated with decreased ICP4 synthesis, was observed.

(Wong, Rose and Chatterjee)

MOLECULAR EPIDEMIOLOGY OF NEWLY RECOGNIZED HERPESVIRUSES

Two previously unknown herpesviruses have been identified in recent years, as agents associated with human T lymphocytes. Human herpesvirus 6 (HHV-6) was first recognized in 1986, by Salahuddin et al. (Lab. Tumor Virology, NCI). This was followed by isolation of additional strains from AIDS patients, including the U1102 strain from Uganda, and the Z29 strain from Zaire. In 1988, HHV-6 was found by Yamanishi et al. to cause exanthem subitum (ES), or roseola infantum. Whereas diseases associated with HHV-6 are currently being unraveled (see below), variations between these viruses has become apparent, as more strains were isolated. It has become of prime importance to determine the relatedness of virus strains, and whether they cause the same or different disease(s).

Molecular epidemiology of HHV-6 Z26-like viruses. Following the isolation of virus strains worldwide, the identification of the new viruses relied mostly on hybridizations with defined viral clones, amounting to a small fraction of the viral genome. To determine the relatedness of viruses we have examined, by different restriction enzymes, the cleavage patterns of the entire virus genome. We have examined multiple ES virus isolates from Japan and the USA (obtained from Yamanishi et al. and from our own laboratory, from ES patients in Children's Hospital, Washington, DC). The ES isolates were all similar to strain Z29, but different from the U1102 strain. Two of six mAbs derived by Balachandran et al. for the GS strain of HHV-6 did not cross react. Most recently, in collaboration with Asano et al. (Japan), we have shown that HHV-6 Z29-like virus was recovered from a number of acute diseases, including: 9 and 13 months old babies with fatal encephalitis, a 2 month old baby with fatal fulminant hepatitis, and 8 renal transplant patients undergoing immunosuppression. Viruses isolated from two of the renal transplant recipients were found to be identical, strongly suggesting that the virus was transmitted from the donor to the two recipients. In addition, we examined 12 isolates from patients following bone marrow transplantation. Repeated isolation revealed long viremia in contrast to ES cases, where virus can only be isolated for 3-4 days at the time of the high fever. In all cases above, the viruses recovered were HHV-6 Z29-like strains. Although still preliminary, it appears that HHV-6 can be associated with morbidity and mortality in the young as well as immunocompromised patients.

(Frenkel, Katsafanas, Wyatt and Asano)

The U1102 like viruses-the case for HHV-8. Our analyses above have all yielded Z29-like viruses (40 strains in all from primary diseases and transplant patients). Recently, we have shown that seven viral strains (obtained from Ablashi, Kruger, Carrigan, Salahuddin and the late R. Honess) were similar to the U1102 prototype. Thus, the previously designated HHV-6 were in two distinct categories: the Z29-like viruses, and the U1102-like viruses. Our proposal to reclassify the HHV-6 strains as two separate herpesviruses is currently in its final stages of international formation. In addition to the restriction enzyme data, it was based on data for three different sets of mAbs, as well as epidemiologically. On the basis of these results, and supported by data of other investigators, we propose that the viruses be designated as ES Virus (ESV) or HHV-6, and HHV-8. HHV-7, previously isolated and characterized by us, remains as a distinct virus (see below). HHV-6, HHV-7 and HHV-8 can be easily distinguished from one another. None of the ES isolates were in the HHV-8 category. All the HHV-8 viruses thus far have been isolated from AIDS patients or from chronic fatigue patients.

(Frenkel, Katsafanas and Wyatt)

Restriction Enzyme Mapping of ESV and HHV-8. In collaboration with P. Pellett in the CDC, we have mapped the restriction enzyme sites of ESV strain Z29, and the DNA of HHV-8 strain U1102. Although all large probes cross hybridized, many of the sites map differently. In all cases, all sites are well conserved within the ESV strains and within the HHV-8 strains. Each of the strains differed in the heterogeneous sequences within the terminal repeat.

(Danovich, Schirmer and Frenkel)

Characterization of HHV-7. HHV-7 remains as a distinct virus as judged by restriction enzyme analyses, hybridization analyses, antigenic reactivity and epidemiological data. We have continued the derivation of approaches to be used in studies of HHV-7. Further cloning and mapping of HHV-7 DNA have been pursued. Sequences of the junction between the direct terminal repeat and the unique sequences have been analyzed. Clones amounting to more than 40 kbp have been derived for HHV-7. PCR primers have been derived and found to be specific for HHV-7.

(Wyatt, Schirmer, Danovich and Frenkel)

Isolation of HHV-7 from saliva. We have shown that a significant fraction of adults (13 of 25 tested) carry cell-free infectious virus in their saliva. The viruses isolated from the same individual over more than 100 days were similar, indicating the occurrence of persistent infection. All 13 saliva isolates were found to react with only 2 of 6 mAbs made against strain GS.

(Wyatt and Frenkel)

Virus-Host Cell Interaction. Our previous studies included analyses of the effect of T cell activation on virus replication. We have recently shown that HHV-6, and HHV-8 cannot be reactivated from latency, in vitro, whereas HHV-7 could be reactivated, by exposing the cells to T cell activation conditions. We have shown that HHV-6 requires helper virus, such as HHV-7, for its reactivation. Entry and reactivation from latency are of medical importance, inasmuch as viral reactivation occurs in organ transplant and bone marrow transplant patients undergoing immunosuppression.

(Katsafanas, Wyatt and Frenkel)

Sensitivity to interferons. We have analyzed HHV-6 sensitivity to interferons. We have shown that in quiescent cells which are coactivated with PHA, at the time of infection, virus replication is sensitive to α and γ interferons. In cells which were preactivated with mitogen prior to virus infection, viral replication was inhibited by α interferon, but was not inhibited by γ interferon, most likely because of modulation of cell surface receptors.

(Avidor and Frenkel)

HHV-6 and HHV-7 as a cofactor in AIDS. In a recent set of studies in collaboration with Dr. Sever of Children's Hospital, we have investigated whether HHV-6 plays a role as a cofactor, in the rapid development of AIDS in children born to HIV+ mothers. Preliminary results, based on limited sampling, have shown that HHV-7 infection coincides with an earlier onset of AIDS in these children. A prospective study has been designed, and will continue in collaboration with Dr. Sever. Also noteworthy are preliminary results that HIV seropositive patients do not develop high titers of antibodies to HHV-6. It is planned that studies designed to investigate these questions will continue.

(Wyatt, Frenkel and Sever)

ADMINISTRATIVE CHANGES

Dr. Niza Frenkel resigned her position as Head of the Molecular Genetics Section of LVD to become a Professor in the Department of Cell Research and Immunology at Tel Aviv University in Israel. Dr. Edwin Sebring, a member of the Molecular Structure Section, retired.

The following trainees or postdoctoral fellows left to take other positions: Bernard Amegadzie - Smith Kline Beecham Pharmaceuticals, Philadelphia, PA; Boaz Avidor - Department of Biological Chemistry, Hebrew University, Jerusalem, Israel; Rafael Blasco, Centro de Investigacion y Technologia del Inia - Sanidad Animal, Madrid, Spain; Wan Chen, Department of Epidemiology, University of North Carolina, Chapel Hill, NC; Robert Danovich, Schering Plough, Scotch Plains, NJ; Lawrence Eisenlohr; Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, PA; Fernando Esquivel, Centro de Investigacion Genetica y Biotechnologia, Cuernavoca, Mexico; Stuart Isaacs, Division of Geographic Medicine & Infectious Disease, Tufts-New England Medical Center, Boston, MA; Brian Lum, University of Chicago, Chicago, IL; Ehud Roffman, Department of Cell Research & Immunology, Tel Aviv University, Tel Aviv, Israel; Kamehameha Wong, City of Hope, National Medical Center, Duarte, CA; Hao Yuwen, Division of Cancer Etiology, NCI.

HONORS, AWARDS AND SERVICE

Mark Challberg received the NIH Directors' Award. Members of LVD received numerous invitations to present major lectures at international and national meetings and at universities. Drs. Challberg, Moss and Rose continue to serve on the editorial boards of one or more scientific journals. In addition, Dr. Bennink is an Associate Editor of the Journal of Immunology and Dr. Moss is an Editor of Virology. Dr. Challberg has served as an advisor to the National Science Foundation and has recently been appointed to an NIH Virology Study Section. Dr. Moss is a member of the National Academy of Sciences and served on the NIH AIDS Vaccine Selection Committee; the NIAID Task Force on Microbiology and Infectious Diseases; the Task Force on the Intramural Research Program of the National Institutes of Health; and the Vaccinia Subcommittee, Interagency Group, National Vaccine Program.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00123-26 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Replication of Poxvirus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. J. Merchlinsky	Expert	LVD, NIAID
Others:	B. Moss	Chief	LVD, NIAID
	A. Levy	Visiting Fellow	LVD, NIAID
	S. Sickafuse	Technician (Micro.)	LVD, NIAID

COOPERATING UNITS *(if any)*

none

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
    _(a1) Minors
    _(a2) Interviews
_(b) Human tissues
_X(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Poxviruses provide a unique system for studying the replication of DNA. Required enzymes and factors are encoded within the viral genome and DNA synthesis and processing occurs within the cytoplasmic compartment of the cell. Therefore, it has been possible to apply genetic and biochemical approaches to the study of DNA replication. Our effort has been towards ascertaining the structure and mode of replication of the poxvirus genome with particular emphasis placed on understanding the processing of the replicative intermediates including the telomere-like hairpin structure and the enymes involved in its replication. The replication of vaccinia virus proceeds through concatemeric intermediates which are resolved into unit length DNA. Mutational analysis has demonstrated that a specific *cis* acting DNA sequence, highly conserved among poxviruses, as well as the palindromic structure of the concatemer junction, is essential for resolution, and that resolution occurred by conservative strand exchange. A model for resolution involving site-specific recombination and orientated branch migration is consistent with this data. We have identified proteins that are candidates for trans-acting components of telomere resolution and are characterizing their properties. A procedure has been developed for the construction of viral genomes containing large inserts of foreign DNA by ligation of subgenomic viral DNA fragments in the presence of the insert DNA. These DNA molecules are transfected into cells where they can be packaged into viral particles and subsequently propagated as virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER
Z01 AI 00126-19 LVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Analysis of Vaccinia Virus DNA Polymerase Gene Alterations

PRINCIPAL INVESTIGATOR Frank M. DeFilippes Research Physicist LVD, NIAID

Others: None

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1	**PROFESSIONAL:** 1	**OTHER:** 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Experiments were designed to test the assumption that the conserved residues (CR) of a protein are essential for its function. Procedures were developed for the efficient use of the polymerase chain reaction (PCR) to introduce alterations into codons for CRs in a region of the vaccinia virus DNA polymerase. These alterations are linked to a mutation in an adjacent site which confers resistance to aphidicolin (AP), a drug which is a competitive inhibitor for cytidine deoxyribonucleotide triphosphate (dCTP). A single priming oligonucleotide with an alteration of a codon for a specific CR and the mutation which confers AP resistance (APr) was used in the PCR to produce an altered DNA which was introduced into virus infected cells by marker transfer (MT). An extract of these cells was diluted and altered virus was detected by plaque formation in AP medium. The most significant substitution to date was the replacement of a pair of amino acids, tyrosine and alanine, by tryptophan and threonine, in the same sequence. Vaccinia virus with these alterations grew at the normal rate and produced a normal yield of plaques. No plaques were detected after marker transfer when more "drastic" substitutions of highly conserved residues, such as substituting aspartic acid for tyrosine, were attempted. This result indicates that within the limits of the MT reaction such substitutions are not allowed. Controls for the MT reaction included a standard substitution which utilizes an oligonucleotide that inserts a BglI restriction site into the region with the CRs. In addition, every priming oligonucleotide which contained an alteration of a CR also contained the alterations to introduce the Bgl site as well as the APr mutation. PCR methods were developed to amplify small amounts of virus DNA from APr plaques to check the sequence of the DNA for the desired alterations.

U.S. GOVERNMENT PRINTING OFFICE. 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00294-11 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Pl:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID

COOPERATING UNITS *(if any)*

R. McPherson, M.D., Department of Pathology, Scripps Clinic, San Diego, CA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.2	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and function of certain sequences (e.g., the inverted terminal repeats) and genes of adenovirus (ad) DNA. In earlier studies, we first identified and characterized the VA RNA gene/transcript and inverted terminal repeats in Ad DNA. We have continued to investigate the specific functions of several early Ad genes, e.g., the VA and DNA-binding protein genes. Our results indicate that these latter genes are involved in the regulation of translation of certain viral mRNAs. Among methods used are gel electrophoresis, gene cloning, mutagenesis, base sequence analysis, and DNA transfection.

PHS 6040 (Rev. 5/92)

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00295-11 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Rose	Section Head	LVD, NIAID

COOPERATING UNITS *(if any)*

L. Mishra, Senior Staff Fellow, Division of Blood and Blood Products, CBER, FDA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of this project are (i) to identify the helper virus genes that regulate expression of defective human parvovirus (AAV) genomes, and (ii) to define their respective roles in AAV replication. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are specific immunofluorescence, DNA cloning, gel electrophoresis, blot-hybridization analyses, and DNA transfection of both simian and human cell lines.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00296-11 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Rose	Section Head	LVD, NIAID
Other:	S. Muralidhar	Visiting Associate	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of these studies are (1) to identify and characterize all proteins that are specified by the defective human parvovirus (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (2) to define the mechanism(s) by which the AAV proteins arise, and (3) to define specific functions of the AAV proteins. We have identified at least four AAV non-structural proteins. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins although they share large segments of sequences-in-common. It is now clear, however, that these proteins originate from independent in-frame initiations. Mechanism that regulate expression of AAV proteins are of fundamental interest, and we have shown that one AAV structural protein is initiated by a codon (ACG) not known previously to act as an initiation codon in higher eukaryotes. Furthermore, our current findings (i) support a "scanning mechanism" in the translational expression of polycistronic eukaryotic mRNAs, and (ii) demonstrate that alternative mRNA splicing is required for effective translational expression of the largest AAV capsid protein. Among methods used are site-directed mutagenesis, affinity chromatography, gel electrophoresis, in vitro translation of viral RNA, DNA transfection, immunoprecipitation, and Western blotting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00297-11 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary objective of this project is to define molecular and biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both in vivo and in vitro systems. We have shown that AAV DNA synthesis can be initiated in vitro in cell-free extracts and that replicating forms that correspond to those identified in vivo can be synthesized with either endogenous or exogenously added templates. Recently, we have shown that AAV "rep" proteins may not be required for the synthesis of AAV concatemeric DNA intermediates, but that one on more of these proteins is required to resolve the long intermediates into unit length duplexes. The observed mode of AAV DNA replication may serve as a model for telomere replication in eukaryotes. Among methods used are affinity chromatography, DNA sequence analysis, and restriction cleavage of DNA molecules and DNA transfection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER
Z01 AI 00298-11 LVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR	B. Moss	Chief	LVD, NIAID
Others:	G. Sutter	Special Volunteer	LVD, NIAID
	S. Isaacs	Medical Staff Fellow	LVD, NIAID

COOPERATING UNITS *(If any)*
M. Brownstein, NIMH; T. Fuerst, MedImmune; R.A. Morgan, NHLBI

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The cytoplasmic site of gene expression and use of virally encoded enzymes is a distinguishing feature of vaccinia virus and other poxvirus vector systems that probably accounts for their consistent ability to express foreign genes derived from a variety of prokaryotic, eukaryotic, and viral sources. This feature, together with their ability to stably integrate and package large amounts of DNA without loss of infectivity, their wide host range, and the development of simple and effective methods for isolating recombinant viruses account for their diverse use and popularity.

Increased expression of genes cloned into the vaccinia virus genome has been achieved primarily through the use of mutated and synthetic promoters. The current levels of expression are up to 50-fold higher than that achieved with the original vectors. An alternative procedure, in which the RNA polymerase and regulatory sequences of a bacteriophage are used, also has been developed. Initially, the transcriptional components of bacteriophage T7 were used and this has now been extended to SP6 thereby increasing the versatility of the system.

Several approaches have been used to develop highly attenuated vaccinia virus vector strains that would be safer in the laboratory and more suitable as live vaccines. The first has been to identify and delete genes that are not required for replication in tissue culture but which contribute to virulence in animals. The effects of deletion of the genes encoding the vaccinia virus complement control protein, which would allow more rapid clearance of the virus by the host and the VP37 envelope protein, which would limit cell-to-cell spread of virus, are currently being tested. Another approach is to evaluate strains of vaccinia virus that were attenuated by extensive tissue culture passage during the era of small pox vaccination. We determined that the highly attenuated MVA strain, which cannot produce infectious virus in mammalian cells, still has the capacity to express foreign genes at a very high level.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00306-11 LVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Pathogenesis of Orthopoxvirus Infections

PRINCIPAL INVESTIGATOR	R. M. L. Buller	Expert	LVD, NIAID
Others:	G. Palumbo	Staff Fellow	LVD, NIAID
	W. Chen	Visiting Fellow	LVD, NIAID
	G. Karupiah	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*
T. Fredrickson, NCI, NIH; D. Pickup, Duke University; T. Eling, NIEHS; Kerry Oliver, NIEHS; R. Drillien, U. of Louis Pasteur, France; E. Brownell, Miles Research Inc.; M. Spriggs, Immunex Research and Development Corp; D. Hruby, Oregon State University

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.75	4	1.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

In this project, we focused our studies on the genetic basis both of poxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute toward development of safe, effective recombinant vaccinia virus vaccines for animal and human use.

Molluscum contagiosum virus infects a large number of HIV-1 patients as an opportunistic infection. The biology of the virus is little understood because it has not been propagated under experimental conditions until now. We have observed virus replication in 25% of the infected human foreskin grafts to the nude mouse. This may provide a model for evaluating therapeutics for treatment of this disease.

The genetic basis for ectromelia virus' narrow host range is poorly understood. Analyses of the tissue tropism genes CHO, K1L and C7L revealed that the CHO gene is inactive and the K1L and C7L genes have only partial activity. Deletion of the K1L gene had no detectable effect on ectromelia virus pathogenesis in the mouse. These observation are consistent with the narrow host-range of ectromelia virus.

A newly identified EV ORF predicted to encode a 28kDa protein was found to replicate normally in tested tissue culture cell lines, but replicated to levels at least 100-fold less than WT EV in the foot-pad of the mouse, spread more slowly from the primary site of inoculation to internal organs and had an LD_{50} that was 1000 times higher than the WT virus. This gene appears to be an important poxvirus virulence determinant in the mousepox model.

The popliteal lymph node which drains the site of virus infection (by the foot-pad route) shows high levels of virus infectivity. Electron microscopy, light level histology and in situ hybridization support the model that the virus replicates in this tissue only transiently up until around 4 days p.i., after which time the lymph node is refractile to infection. The infectivity that is detected in the lymph node is most likely virus which has been carried as free virus in the lymph or in cells trafficking out of the tissue.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH**	**PROJECT NUMBER** Z01 AI 00307-11 LVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR	B. Moss	Laboratory Chief	LVD, NIAID
Others:	B. -Y. Ahn	Visiting Associate	LVD, NIAID
	C. J. Baldick	Pre-IRTA	LVD, NIAID
	P. Gershon	Visiting Associate	LVD, NIAID
	J. Keck	Staff Fellow	LVD, NIAID
	N. Harris	Visiting Fellow	LVD, NIAID
	R. Rosales	Visiting Fellow	LVD, NIAID
	B. Schnierle	Volunteer	LVD, NIAID
	L. Carroll	IRTA	LVD, NIAID
	G. Kovacs	IRTA	LVD, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	**PROFESSIONAL:**	**OTHER:**
8.7	8.2	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Poxviruses, unlike other DNA viruses, replicate in the cytoplasm of the cell and encode most or all of the enzymes and factors needed for transcription of their genomes. Studies with vaccinia virus indicated that the genes are divided into three temporal classes - early, intermediate, and late - that are regulated in a cascade fashion. Vaccinia virus provides a unique system, for combining genetic and biochemical approaches for investigating mechanisms of gene regulation, that has resulted in many basic discoveries.

Further progress was made in defining the viral proteins involved in transcription and in understanding their roles. During the past year, two additional RNA polymerase subunit genes *rpo18* and *rpo7*, encoding polypeptides of 18,000 and 7,000 Daltons, were located within the vaccinia virus genome. The smaller one appears to be homologous to the smallest subunit of eukaryotic RNA polymerases. There are 8 different core subunits known to be present in the RNA polymerase. In addition, a 94,000 Dalton RNA polymerase specificity factor, named rap94, was found to be associated with the transcriptionally active RNA polymerase and shown to be required for transcription.

A detailed analysis of the structure of intermediate class promoters indicated they are composed of a 14 base pair core sequence that is rich in adenosine and thymidine residues separated by one turn of the double helix from a TAAA initiator element. These structural features distinguish intermediate promoters from both early and late promoters and provide a basis for regulated gene expression.

Vaccinia virus encodes enzymes for modifying both ends of mRNA. This past year, the genes encoding the two subunits, VP39 and VP55, of the heterodimeric poly(A) polymerase were identified. VP55 was found to be the catalytic subunit whereas VP39 stimulated elongation of poly(A) tails. VP39 was also shown to be methyltransferase that methylates the cap structure of mRNA which is required for translation.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER
Z01 AI 00416-09 LVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Recombinant Vaccines against Retrovirus Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR	B. Moss	Laboratory Chief	LVD, NIAID
Others:	P. Earl	Microbiologist	LVD, NIAID
	V. Karacostas	Pre-IRTA	LVD, NIAID
	E. Wolffe	Special Volunteer	LVD, NIAID

COOPERATING UNITS ***(if any)***
J. Berzofsky, NCI; UpJohn Company

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	**PROFESSIONAL:**	**OTHER:**
2.7	2.2	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK ***(Use standard unreduced type. Do not exceed the space provided)***

Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS). At present there is no effective vaccine against this disease and therapeutic agents provide only limited help. The objects of this project are to characterize HIV antigens, determine the targets of humoral and cell-mediated immunity, and to use this information to develop candidate vaccines. We have constructed recombinant vaccinia viruses containing HIV genetic information. These viruses have been used as live experimental vaccines to immunize animals, to synthesize HIV proteins in tissue culture, to make targets for cytotoxic T cells, and study CD4-envelope protein interactions.

Our previous studies demonstrated that the HIV envelope protein exists as dimers and tetramers. The question remained, however, whether the envelope protein oligomers could bind multiple CD4 molecules. We have now shown that the dimers, but not the monomers, can bind two CD4 molecules simultaneously. Multimeric CD4 binding may have important implications for env-protein CD4 avidity, CD4-induced release of gp120, subunit-subunit cooperativity during virus membrane fusion, as well as therapeutic strategies.

The consequences of altering the ratios of the HIV-1 Gag and Pol proteins were tested by making a single nucleotide insertion that placed the gag and pol genes in the same open reading frame. This alteration led to overproduction of pol gene products including reverse transcriptase and protease and resulted in failure to assemble and bud particles. The block to assembly was partially overcome by inhibition of the protease with a peptidomimetic inhibitor, mutagenesis of the active site of the protease, or shortening of the Gag-Pol polyprotein. These studies provide important insights into the mechanism of HIV particle formation.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-930

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER
Z01 AI 00445-08 LVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Mechanisms of Viral DNA Replication

PRINCIPAL INVESTIGATOR	M. D. Challberg	Section Head	LVD, NIAID
Others:	D. Fierer	Medical Fellow	LVD, NIAID
	J. Gottlieb	IRTA Fellow	LVD, NIAID
	G. Sherman	IRTA Fellow	LVD, NIAID
	D. Klinedinst	IRTA Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.1	5.0	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐(c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Herpes simplex virus is a useful model for studying the mechanisms involved in DNA replication in eukaryotic cells. Our current efforts are directed toward studying this process with the purified protein products of the seven viral genes that are necessary to carry out authentic origin-dependent DNA replication in infected cells. We are currently using both biochemical and molecular genetic approaches to understand the function of these polypeptides in detail.

The HSV DNA polymerase consists of a stable complex of two polypeptides: UL30, the catalytic subunit, and UL42, an accessory subunit. Several lines of evidence support the view UL42 increases the efficiency of the DNA polymerase by increasing its processivity. We have begun to dissect the function of the UL42 processivity factor using directed mutagenesis. Our results to data reveal that the carboxyl terminal third of the polypeptide is not essential for any function in DNA replication. Additional mutants that may shed some light on the mechanism of UL42 and the regulation of processivity are currently are under investigation.

The UL5, UL8, and UL52 polypeptides form a three protein complex that has both helicase and primase activities. We have proposed that UL52 has the active site for primase function, and are trying to obtain direct support for this view. Several mutants of UL52 that have missense mutations in the most highly conserved regions of the protein have been isolated. At least three of these mutants do not support DNA replication, even though the mutant polypeptide is capable of forming a heterotrimeric complex with UL5 and UL8. The biochemical defect of these mutants is now being determined.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-839

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH**	**PROJECT NUMBER** Z01 AI 00538-05 LVD

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT
Interaction of Human Immunodeficiency Virus with the CD4 Receptor

PRINCIPAL INVESTIGATOR	E. Berger	Expert	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID
	C. Broder	NRC Associate	LVD, NIAID
	O. Nussbaum	Visiting Associate	LVD, NIAID
	P. Kennedy	Microbiologist	LVD, NIAID

COOPERATING UNITS *(if any)*
R. Blumenthal, D. Dimitrov, NCI
I. Pastan, D. FitzGerald, NCI

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	**PROFESSIONAL:**	**OTHER:**

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

CD4 is a surface molecule that serves as the receptor for human immunodeficiency virus (HIV), the causative agent of AIDS. We have focused on two areas related to CD4 and HIV: 1) Mechanisms of membrane fusion mediated by the interaction of CD4 with the HIV-1 envelope glycoprotein (env). One goal of this work is to identify regions of each molecule involved in fusion, and to characterize fusion-related changes in env structure induced upon interaction with CD4. Site-directed mutagenesis studies indicated that CD4 molecules containing a wide variety of mutations in the CDR3 region efficiently support membrane fusion. Mutation of this region also had no effect on the ability of soluble CD4 to induce a specific structural change in env (gp120 stripping). These results challenge the prevailing notion that the CDR3 region plays a critical role in the fusion process. Furthermore, a region of gp120 (V3) believed to participate in fusion appears not to be involved in CD4-induced gp120 stripping, based on studies with fusion-defective V3 mutants. A second goal is to identify additional molecular components involved in fusion. We have demonstrated that human cells can provide to CD4-expressing non-human cells a component(s) which is essential for env-mediated membrane fusion. The critical involvement of calcium ions in the fusion process has also been demonstrated. We are actively developing new systems and assays to study the fusion process, to be applied towards mechanistic studies of membrane fusion and the selective tropism of different HIV isolates for different CD4+ cell types.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE. 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH

PROJECT NUMBER

Z01 AI 00539-05 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT

Virus-Cell Interactions, Viral Pathogenesis, and Host Immunity

PRINCIPAL INVESTIGATOR	B. Moss	Laboratory Chief	LVD, NIAID
Others:	S. Isaacs	Medical Staff Fellow	LVD, NIAID
	R. Blasco	Visiting Associate	LVD, NIAID
	Y. Zhang	Visiting Fellow	LVD, NIAID
	E. Wolffe	Volunteer	LVD, NIAID

COOPERATING UNITS *(If any)*

M. Frank and R. McKenzie, LCI, NIAID
R.W. Doms, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	3.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

A successful virus infection involves entry into the cell; uncoating, expression and replication of the genome; assembly and release of infectious virus particles; and defense against specific and non-specific host immune mechanisms. Combined genetic, biochemical, electron microscopic, and immunological approaches are being used to investigate these complex processes.

Two genetic approaches have been developed and applied to the study of vaccinia virus assembly. For essential genes, the elements of the E. coli lac operon were used to construct inducer-dependent conditional lethal mutants of vaccinia virus. Repression of the D13L gene, which encodes a 65,000 Dalton protein, prevented the formation of the immature spicule-coated viral envelope and mimiced the action of the drug rifampicin. Represssion of the F18R gene allowed virion morphogenesis to proceed up to the stage of differentiation of the virion core. The second approach, gene knock-out, was used to investigate the formation of the outer viral envelope and the release of virions from cells. Deletion of the viral gene encoding the VP37 envelope protein did not affect the formation of intracellular infectious virus but prevented the membrane wrapping of virions and their fusion with the plasma membrane. Evidence was obtained for an additional post-budding step in virion release. With some strains of vaccinia virus, enveloped virions remains attached to the surface of cells but can be released with trypsin treatment.

To evade the host immune system, vaccinia virus encodes a variety of proteins. We demonstrated that the complement control protein (VCP), the secreted product of the C2IL gene, is able to prevent complement-enhanced antibody-mediated neutralization of infectious virus. These data demonstrated that VCP blocks the alternative, as well as the classical, pathway of complement activation. A mutant virus containing a knock-out deletion of the C2IL gene was highly attenuated when injected into the skin of animals. The basis for the interferon-resistance of vaccinia virus was investigated. The product of the K3L gene was found to inhibit the double-stranded RNA activated protein kinase and to thereby prevent the interferon induced phosphorylation of the alpha subunit of eukaryotic intitiation factor 2. Evidence agtainst the possibility that vaccnia inhibits host protein synthesis by degrading or causing the underphosphorylation of components of the cap-binding translation initiation factor complex eIF-4F was obtained.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-664-938

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00540-05-LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Human Herpesviruses 6 and 7

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Frenkel	Head Section	LVD, NIAID
Others:	L. Wyatt	Senior Staff Fellow	LVD, NIAID
	B. Avidor	Visiting Associate	LVD, NIAID
	E. Roffman	Visiting Associate	LVD, NIAID
	B. Lum	IRTA Fellow	LVD, NIAID
	R. Danovich	NRC Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

Dr. D. Di Luca, University of Ferrara, Italy.
Dr. J. Sever, Children's Hospital, Washington, DC.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This report concerns the structure and function of human herpesviruses 6 and 7, their interactions with HIV, and interactions with T cells. We have recently shown that virus strains previously recognized as HHV-6, isolated in the USA, Europe, and Japan, fall into two distinct categories. These virus groups have been tentatively designated as Exanthem Subitum Virus (or HHV-6) and HHV-8. HHV-7 was isolated in our laboratory in 1989, from CD4+ T cells of a healthy individual. Our studies this past year concerned several topics: (i) we have continued the derivation of approaches to be used in studies of HHV-7. Further cloning and mapping of HHV-7 DNA has been pursued. Sequences of the junction between the direct terminal repeat and the unique sequences have been analyzed. PCR primers have been derived and tested for specificity. (ii) We have continued our studies relevant to virus-host cell interactions. Our previous studies included analyses of the effect of T cell activation on virus replication, activation of HHV-6 and HHV-7 from latency, the effect of virus infection on T cell function, and analyses of parameters which determine entry into latency. We have analyzed HHV-6 sensitivity to interferon. We have shown the conditions for activation of HHV-6 and HHV-7 from latency. Entry and reactivation from latency are of potential medical importance, in as much as viral reactivation occurs in organ transplant and bone marrow transplant patients undergoing immunosuppression. (iii) In a study currently in progress, in collaboration with Dr. D. DiLuca of University of Ferrara, we have investigated the prevalence of HHV-7 DNA in Hodgkin's and non-Hodgkin's lymphomas. (iv) In recent studies, in collaboration with Dr. Sever of Childrens' Hospital, we have investigated whether HHV-6 plays a role as a cofactor, in the rapid development of AIDS in children to HIV+ mothers. Preliminary results have been obtained and future prospective studies have been designed. (v) We are attempting to characterize the microenvironment of the CD4 molecule on T cells. Further studies are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00541-05 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Folding, Assembly, and Transport of Viral Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
	J.R. Bennink	Microbiologist	LVD, NIAID

COOPERATING UNITS *(if any)*

T. Bachi, Electron Microscopy Laboratory, Zurich, Switzerland
N.P. Hauri, Biozentrum, Basel, Switzerland

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.44 staff year	0.22 staff year	0.22 staff year

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The improvement of current anti-viral vaccines and the development of novel vaccines depends on increasing our understanding of viral attachment and fusion glycoproteins. Critical insight into understanding the antigenic structure of glycoproteins is provided by studying their interaction with monoclonal antibodies (mAbs). For a number of years we have studied the influenza virus hemagglutinin (HA) glycoprotein. This protein serves as a model for other proteins with similar functions (e.g. HIV gp160), and moreover, is important practically in its own right, as influenza still is a major cause of morbidity and mortality both nationally and internationally.

Like many viral glycoproteins the HA is a homo-oligomer, consisting of three identical monomeric subunits. In the past year we continued to investigate the interaction of HA with a particular mAb that recognizes an antigenic site that is hidden when the HA trimerizes. We found that this antibody is able to interact with trimers when incubated at body temperature (37 °C) or above, and that this reactivity reflects the conformational "breathing" of the tip of the HA, which allows antibody access to a location in the molecule that is inaccessible at lower temperatures. Surprisingly, this "breathing" was restricted by mutations in the HA located near the base of the molecule. These findings indicate that the interaction of viral glycoproteins with protective antibodies can be influenced by very subtle changes in the dynamics of the quaternary structure of the viral protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00542-05 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Processing of Viral Proteins for T Cell Recognition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.R. Bennink	Microbiologist	LVD, NIAID
	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
Others:	Fernando Esquivel	Visiting Fellow	LVD, NIAID
	Patricia Day	IRTA Fellow	LVD, NIAID
	Cheryl Lapham	IRTA Fellow	LVD, NIAID
	Yuping Deng	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

Jan Lukso, BRB, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.44 staff years	.22 staff year	2.22 staff year

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Class I molecules of the major histocompatibility complex (MHC) consist of a highly polymorphic heavy chain complexed to beta$_2$ microglobulin (beta$_2$m). Class I molecules are expressed on virtually all cell types. Their sole function is to bind antigens and present them to T cell bearing CD8 molecules (T_{CD8+}). T_{CD8+} play a critical role in eradicating intracellular pathogens and tumors. They are also a significant cause of immunopathology, being involved in organ rejection and autoimmune diseases.

There has been rapid progress in understanding the physical nature of the antigen-class I complex and in how antigens are generated and become associated with class I molecules in cells. It is now apparent that antigens derived from a cytosolic pool of proteins are translocated into an exocytic compartment by a MHC encoded transporter complex. Once transported from the cytosol, antigens bind to class I molecules and are transported to the cell surface.

Continuing our studies on the assembly and trafficking of MHC class I molecules in the past year we:

1) obtained the first direct evidence that peptides associate with class I molecules in an early exocytic compartment,

2) genetically engineered a class I molecule retained in the endoplasmic reticulum (ER) that will provide finer resolution into the identity of the compartment in which antigen association occurs,

3) developed a fluorescent derivative of an antigenic peptide that retains the capacity to bind class I molecules and offers the promise of studying class I assembly and transport in living cells,

4) found that cells differentially internalize from the cell surface two different types of class I molecules, and made preliminary findings that suggest that cell surface class I molecules can be returned to the Golgi complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00564-04 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Parvovirus Vectors that Regulate Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. Rose	Section Head	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was designed to develop adeno-associated virus (AAV)-based vectors that could down-regulate expression of specific viral genes by mediating the production of appropriate antisense RNA molecules. Among targets chosen for study are certain early viral gene products which are absolutely necessary for productive viral infection. A series of AAV-based vectors has been created in which the endogenous AAV promoters and coding sequences were replaced with a polylinker and several strong constitutive and inducible heterologous promoters. An encapsidation system has been developed in orders to produce high titres of infectious recombinant virus. The induction of resistance to both HIV-1 infection by such vectors is currently being studies, and present results indicate that a 95% reduction in the replication of either viruses can be achieved. Methods used include: gene cloning, transfection of cells, radio-innunoprecipitation, immunofluorescence, RNA and DNA hybridization techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00619-02 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intracellular Antibody-mediated virus neutralization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
	J.R. Bennink	Microbiologist	LVD, NIAID
Others:	Rosemary Ffrench	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.33 staff years	.22 staff year	1.1 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the greatest hurdles in creating vaccines for a number of viral pathogens is the antigenic variability of viral coat proteins. The rapid mutation rate of a number of viruses (most notably influenza virus and HIV) allows the virus to escape the neutralizing antibody response induced by anti-viral vaccines. The internal proteins of the virus, on the other hand, are generally highly conserved, and have not evolved to rapidly alter their antigenicity. Antibody responses to these internal proteins, while a consistent feature of immune responses to viruses, almost always fail to influence viral infectivity, since the antibodies do not have access to their target antigens, which are located either inside the virus, or inside the virus infected cells. If, however, antibodies are introduced into the cytosol of cells, they do have the ability to prevent viral infection. Recent advances in understanding antibody folding indicate that antibodies have the ability to properly fold and bind antigen when they are expressed in the cytosol by removing their amino terminal ER insertion sequences. Therefore, cells, or eventually, transgeneic animals expressing such cytosolic antibodies should be resistant to virus infection. Using electroporation to introduce antibodies into the cytosol, we identified antibodies able to block infection of cells with influenza viruses. In the past year we have cloned the heavy and light chain genes from a hybridoma secreting an antibody that is able to block infection with any of the human influenza A virus subtypes. After removing the portion of the genes encoding the ER insertion sequence, we have inserted these gene into eukaryotic expression vectors, and have transfected cells with the vectors. We are in the process of characterizing the expression and folding of these cytosolic antibodies, and the resistance of antibody expressing cells to influenza A virus infection. Our goal is to create transgenic animals resistant to influenza virus infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00620-02-LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Epidemiology of Human Herpesviruses 6 and 7

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Frenkel	Section Head	LVD, NIAID
Others:	L. Wyatt	Senior Staff Fellow	LVD, NIAID
	G. Katsafanas	Biologist	LVD, NIAID
	T. Butler	Biologist	LVD, NIAID

COOPERATING UNITS *(if any)*

Y. Asano. Fugita Health University, School of Medicine, JAPAN

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns the new lymphotropic human herpesviruses 6 (HHV-6), HHV-7 and HHV-8. The overall goal of these studies has been to determine virus involvement in human disease(s). The results of these studies during the past year can be summarized at follows: (i) HHV-6 and HHV-8. Characterization of multiple virus isolates have revealed that the herpesviruses previously designated as HHV-6 fall into two distinct categories. These viruses differed in restriction enzyme pattern of viral DNA, antigenically and epidemiologically. As per these studies, and confirmed by others, these viruses will be classified as Exanthem Subitum Virus (ESV), prototyped by the HHV-6 Z29 strain, and HHV-8, prototyped by the U1102, or by the GS strains. (ii) Disease association of ESV or HHV-6. In addition to viruses from ES patients, we have also analyzed viruses from patients with fatal encephalitis and fatal fulminant hepatitis. All of these viruses were similar to ESV strain Z29. They were distinct from the U1102 strain, as well as from HHV-7 DNA. (iii) Virus reactivation from latency: Analyses of viruses from 8 renal transplant patients and 10 bone marrow transplant patients revealed similarity to the Z29 virus, except minor strain variations in the terminal repeat sequences. In one case of renal transplant the reactivated virus originated from the donor kidneys, in as much as identical viruses were derived from two transplanted kidneys from the same donor. In ES patients, virus can only be recovered during 3-4 days of the acute (high fever) phase, in BMT patients virus could be repeatedly isolated for lengthy time, revealing long term viremia. We conclude that ESV (rather than HHV-8), can be associated with morbidity and mortality in immunocompromised patients. Further studies are designed with additional clinical centers, in the USA, Japan and Israel, to extend these conclusions, as well as to allow early detection of reactivated virus, for more timely therapy with potent anti-viral drugs which are too toxic for lengthy therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00652-01 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Peptide Transporters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.R. Bennink	Microbiologist	LVD, NIAID
	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
Others:	F. Esquivel	Visiting Fellow	LVD, NIAID
	I. Ba´ik	Visiting Fellow	LVD, NIAID
	L. Eisenlohr	Staff Fellow	LVD, NIAID
	R. Anderson	NRC Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

T. Spies, Dana Farber Cancer Institute, Boston, MA
R. Demars, University of Wisconsin, Madison, WI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.44 staff years	0.22 staff year	2.22 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8+ T cells (T_{CD8+}) play an important role in controlling virus infections. T_{CD8+} recognize peptides of 8 to 10 residues derived from viral proteins located in the cytosol of virus infected cells. These peptides are recognized in a complex with class I molecules encoded by the major histocompatibility complex (MHC). In the past 2 years it was discovered that the MHC also encodes two molecules, termed Tap1 and Tap2, that seem to specifically transport peptides from the cytosol into the intracellular compartment that contains class I molecules. Tap genes have been found to be polymorphic in humans, although to a much lesser extent than the class I molecules. The existence of these putative peptide pumps and their polymorphism raises a number of important questions: Where in the cells are the pumps located? Do the pumps influence the types of peptides presented by class I molecules? Are the pumps tethered to the proteases that produce antigenic peptides in the cytosol? How do the pumps work? Are there individuals with immune deficiencies based on mutations in the Tap genes? Can cells transport peptides via other mechanisms?

To help characterize the structure and function of the Tap genes we have inserted them into vaccinia virus. We have demonstrated that a Vac recombinant encoding Tap1 produces a fully functional gene, and have begun the biochemical and immunocytochemical characterization of the gene. We have just begun characterization of a vac recombinant that contains the Tap 2 gene. Additionally, we have examined the specificity of the antigen transporters. We found that the transporters can transport the exact peptides presented by class I molecules. Also we examined whether expression of the human Tap2 gene in mouse cells adversely affects the ability of the cells to present viral proteins to T_{CD8+}. We found that the human Tap2 gene functions in a indistinguishable manner from its mouse counterpart. Finally, we explored the presentation of peptides in cells that fail to express one or both Tap genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00653-01 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Delivery of antigens to the MHC class I Processing Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.R. Bennink	Microbiologist	LVD, NIAID
	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
Others:	R. Anderson	NRC Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

C. Leamon, Purdue University, W. Layfayette, IN
P.Low, Purdue University, W. Layfayette, IN

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.94 staff year	0.22 staff year	0.72 staff year

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8 positive lymphocytes (T_{CD8+}) play an important role in host immunity to viruses and other intracellular parasites. Anti-viral T_{CD8+} recognize peptides derived from a cytosolic pool of viral proteins. Induction of anti-viral T_{CD8+} responses is limited by the requirement that the antigen be delivered to the cytosol of antigen presenting cells in vivo. Using infectious vaccines, this is accomplished as part of the infectious cycle of the immunogen. Non-infectious vaccines (inactivated virus, subviral preparations) often fail to induced T_{CD8+} responses due to their inability to deliver antigens to the cytosol. It should be possible, however, to induce T_{CD8+} responses to non-infectious antigens, if the antigen is modified in such a way as to allow its efficient delivery to the cytosol. To explore this vaccine strategy, we studied a model antigen (ovalbumin) that we chemically conjugated to folic acid. By targeting proteins to folic acid receptors, it is believed that this modification facilitates the delivery of proteins to the cytosol. In the past year we have studied the presentation of folic acid-conjugated ovalbumin to ovalbumin-specific T_{CD8+}. Our preliminary findings suggest that this strategy might be useful for induction of T_{CD8+} responses to simple protein antigens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00658-01 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Proteolytic generation of antigenic peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
	J.R. Bennink	Microbiologist	LVD, NIAID
Others:	Josephine Cox	Visiting Scientist	LVD, NIAID
	Igor Bacik	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

P. Kourilsky, Institut Pasteur, Paris, France
J.P. Abastado, Institut Pasteur, Paris, France

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.94 staff years	0.22 staff year	1.72 staff year

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CD8 expressing T cells (T_{CD8+}) play a critical role in eradicating intracellular parasites such as viruses. T_{CD8+} recognize MHC class I molecules in a complex with peptides of 8 to 10 residues derived from viral proteins located in the cytosol. There is tremendous interest in the mechanism by which peptides are generated. There is indirect evidence that implicates proteasomes in the generation of antigenic peptides. Proteasomes are abundant macromolecular structures present in the cytosol and nucleus in cells with multiple protease acitivities. They are thought to be responsible for the energy dependent proteolysis in which ubiquitin plays a prominent role in targeting proteins for destruction. Although it is believed that at least some proteolysis occurs in the cytosol, it is uncertain whether the ultimate determinants are generated in the cytosol, or whether additional trimming occurs following transport from the cytosol.

We have initiated several approaches to assess the site and nature of proteolytic mechanisms utilized in the generation of antigenic peptides. First, we produced vaccinia recombinants expressing a form of ubiquitin altered to interfere with ubiquitin-targeted proteolysis. Second, we engineered a cytosolic form of a class I molecule to that should serve as a probe for the presence of antigenic peptides in the cytosol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00659-01 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen Presentation by Human Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.R. Bennink	Microbiologist	LVD, NIAID
	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
Others:	F. Esquivel	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

N. Restifo, Surgery Branch, NCI
S. Rosenberg, Surgery Brach, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.72 staff year	0.22 staff year	0.50 staff year

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is a considerable body of evidence that CD8+ T lymphocytes (T_{CD8+}) are induced by tumors and can function to kill tumor cells. Nevertheless, in many cases, tumors obviously overcome any tumor specific T_{CD8+} response that might be induced following tumor induction, since cancer is a leading cause of death. We are investigating the possibility that in some of these cases, the failure of T_{CD8+} to prevent malignancies is due to the selection of mutants that are unable to effectively present tumor antigen for recognition by the immune system.

In the past few years there has been tremendous progress in our understanding of how cells present antigens for recognition by T_{CD8+}. It is now clear that presentation of antigen results from a chain events including protein breakdown into peptides of 8 to 10 amino acids, transport of peptides from the cytosol to a secretory compartment, binding of peptides to a special peptide-presenting complex, and delivery of the presenting molecule to the cell surface. We devised a rapid and simple method for measuring the ability of tumor cells to present antigens for T_{CD8+} recognition, and found that a high percentage of human tumors were unable to present antigens to T_{CD8+}. We showed that in at least some cases, this is due to the down regulation of all of the known components of the antigen processing and presentation mechanism, and in others, is due to a selective deficiency in a single molecule that forms part of the final peptide presenting complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00660-01 LVD

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Brefeldin A Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
	J.R. Bennink	Microbiologist	LVD, NIAID
Others:	Yuping Deng	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

R. Haugland, Molecular Probes, Eugene, OR
H.C. Kang, Molecular Probes, Eugene, OR

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.94 staff year	.22 staff years	.72 staff years

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Brefeldin A (BFA) is a fungal metabolite with a broad range of biological activities, including the anti-fugal and anti-tumor activities. In the past few years there has been tremendous interest in its effect on cells, since it has been found to have the unique ability to interfere with vescicular trafficking. Among its effects, it has the ability to disperse the Golgi complex, returning its components to the endoplasmic reticulum (ER), and prevent newly synthesized proteins destined for the cell surface from leaving the ER. To better understand the mechanism of BFA action we conjugated it to two fluorescent dyes and studied its intracellular localization. Both conjugates maintained their biological activity, and both specifically localized to the ER in live or aldehyde-fixed cells. The interaction with the ER-membrane is probably due to interaction with lipids, and not with ER proteins. These conjugates are the first dyes that bind the ER without binding other prominent membrane bound compartments, and should prove useful as probes for ER structure in living cells under various conditions. Their ability to stain the ER of fixed cells will be of great use as markers of the ER in cytochemcial studies. Finally, the interaction of BFA with the ER might be critical to its biological activity.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00661-01

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Confocal Microscopy of Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	J.W. Yewdell	Medical Officer (Research)	LVD, NIAID
	J.R. Bennink	Microbiologist	LVD, NIAID

COOPERATING UNITS *(if any)*

R. Germain, LI, NIAID
P. Matzinger, LCMI, NIAID
L. Staudt, MET, NCI and K. Kelly, LP, DCBD, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.44 staff year	PROFESSIONAL: 0.22 staff year	OTHER: 0.22 staff year

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent advances in optics and electronics have culminated in the development of the laser confocal scanning microscope (LCSM). The LCSM provides unparalleled resolution for localizing fluorescent probes in cells. In combinantion with computers, it is relatively easy to produce 3-dimensional reconstructions of fluorescently labelled structures in cells. In the past year we installed a state of the art BioRad MRC 600 LCSM with its controlling computer. We also installed a Silicon Graphic Indigo computer to facilitate manipulation of 3-dimensional data sets generated by the LCSM. We have begun to use this system for a wide variety of applications, including determining the subcellular distribution of proteins, following the intracellular trafficking of newly synthesized membrane proteins, and studying the migration of developing thymocytes in thymic explants.

LABORATORY OF VECTORS AND PATHOGENS
Rocky Mountain Laboratories
Hamilton, Montana
1992 Annual Report
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ANNUAL REPORT
LABORATORY OF VECTORS AND PATHOGENS
HAMILTON, MONTANA
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1991, TO SEPTEMBER 30, 1992

Members of the Laboratory of Vectors and Pathogens have continued their molecular dissection of important human pathogens during the past year. This multidisciplinary approach to microbial pathogenesis combines the fields of biochemistry, immunology, electron microscopy, medical entomology and molecular biology into a cohesive unit with strong interaction among its own members and with scientific units both inside and outside of the NIH system. This interaction together with an active seminar and guest researcher program insures that the best experimental approaches available are rapidly incorporated into the research programs of the laboratory. Since Lyme borreliosis is now the most common arthropod-borne disease in the United States, the Laboratory has made a major commitment of resources to the development of improved diagnostics and to a detailed understanding of how *Borrelia burgdorferi* produces its long list of both acute and chronic clinical features. The Laboratory of Vectors and Pathogens functions in three major experimental groups. Dr. Claude F. Garon serves as Laboratory Chief.

The Arthropod-borne Diseases Section, under the direction of Dr. Tom G. Schwan, is involved is numerous investigations relating to the molecular biology, infectivity, pathogenicity, antigenicity, and immunogenicity of *B. burgdorferi.* The history of Lyme serology dates back only 10 years to 1982 when the first test, an indirect fluorescent antibody (IFA), was reported using the newly discovered spirochete. However, the lack of specificity in the IFA and ELISA using whole *B. burgdorferi* cells has prompted them to search for components of the bacterium that can be used as diagnostic antigens that will improve the specificity and sensitivity at detecting antibodies to *B. burgdorferi.* Previously, the group cloned and expressed a 39 kDa protein of the Lyme spirochete and demonstrated its reactivity with serum from Lyme patients. Recently, additional bacteria were examined to further clarify the specificity of this protein to only Lyme spirochetes. SDS-PAGE and immunoblotting were used to examine bacteria and convalescent human serum samples. Five serovars of *Leptospira interrogans*, *Leptospira biflexa*, *Leptonema illini*, and *Rickettsia rickettsii* were examined and found not to contain the 39 kDa protein of *B. burgdorferi.* Previous studies by others had demonstrated that a small proportion of serum samples from human cases of leptospirosis and Rocky Mountain spotted fever reacted positively with whole cells of *B. burgdorferi.* The specificity of the P39 antigen and its reactivity with human Lyme disease sera should exclude the possibility of false-positive serum samples from patients having had either leptospirosis or Rocky Mountain spotted fever, as well as tick-borne relapsing fever and syphilis. The group has continued its characterization of isolates of *B. burgdorferi* from North America and Eurasia. They have compared the isolates by examining them for numerous molecular determinants with special interest in the P39 antigen. To date they have examined approximately 80 isolates of *B. burgdorferi* from humans, ticks and wild mammals across North American, Europe and Asia. All but one, isolate Ip90 from an *Ixodes persulcatus* tick from the former Soviet Union, expressed this protein. The P39 locus in *B. burgdorferi* is a chromosomal operon encoding two nearly identical size proteins with about 53% amino acid sequence similarity. They have used PCR to begin an analysis of the two genes of this operon in numerous isolates, some of which express a P39 antigen(s) of different size. Most variability appears to be restricted to the first gene (P39α) while the second gene (P39B) appears to be highly conserved. Analysis of strains has included 31 isolates of *B. burgdorferi* cultured from pools of *I. pacificus* ticks collected from numerous regions spanning most of the state of California where this tick is found. Analyses demonstrated that all expressed the P39 protein. Additionally, a rabbit polyclonal antibody they developed showed that most of these isolates expressed some amount of the OspC protein, however, considerable heterogeneity was evident in this protein. (Schwan, Hinnebusch)

Also participating in research on the molecular biology of *Borrelia* is the Structural Pathobiology Section under the direction of Dr. Claude F. Garon. Previous work showed that outer surface protein A (OspA) and OspB of *Borrelia burgdorferi* may occur within an extracellular multiprotein complex, which was resolved by electrophoresis as an 83 kDa major extracellular protein band. To characterize the 83 kDa band, the N terminus of the predominant peptide in the band was sequenced and the interaction between the associated proteins examined. Peptide sequence and amino acid composition comparisons showed identify with the heavy chain of immunoglobulin M (IgM). Reduction sensitivity experiments and the recognition of the band by antibodies specific for rabbit μ chain indicated that the multiprotein complex contained pentameric IgM. Immunoelectron microscopy showed that anti-μ chain antibodies and monoclonal antibodies to OspA and OspB bound to extracellular amorphous material surrounding cells. Furthermore, the Osps coprecipitated with either nonspecific polyclonal rabbit IgM antibodies or with murine monoclonal anti-human serum albumin IgM antibodies, using insoluble anti-μ chain antibody conjugates. Although the apparent 83 kDa complex was stable under conditions of chelation and concentrated salts, it was disrupted by treatment with neuraminidase. These results indicate that extracellular *B. burgdorferi* proteins, including OspA and OspB, interact with IgM. The association is apparently not a classic antibody-antigen interaction but may result from other mechanisms. Significant genetic divergence among isolates was first suggested by differences in monoclonal antibody reactivity, plasmid profiles, and the clinical manifestations of Lyme disease in Europe versus North America. As a result of these observations, considerable emphasis has been focused on the comparison of European and North American isolates. In general, European isolates exhibit greater heterogeneity than North American isolates with respect to several criteria, including monoclonal antibody reactivity and amplification with various polymerase chain reaction (PCR) primers. In a survey of 12 different isolates, including both European and North American representatives, Postic and coworkers identified two genomic groups of DNA-DNA hybridization studies and Southern analysis of rRNA gene restriction patterns. These two genomic groups were termed the *sensu stricto* and 20047 groups. North American isolates reside solely in the *sensu stricto* group. Most European isolates reside in the 20047 group, but some exceptions have been observed. Recently, members of this section investigated the phylogenetic divergence among isolates by using rRNA sequence analysis and rRNA gene restriction profiles. The 16S rRNA sequences were determined for several *B. burgdorferi* isolates as well as for *B. hermsii, B. coriaceae*, and *B. anserina. B. burgdorferi* isolates from Europe, North America, and Russia were included. All isolates from North America (B31, Sh-2-82, and 1352) and isolate 20004 from Europe were found to reside in the *sensu stricto* group. The remaining European isolates, G2 and G1, were placed in the 20047 group. Recently a third genomic group (group 3) comprising tick isolates (*I. persulcatus*) from the Soviet Union was defined. Alignment of the 16S rRNA sequences revealed the presence of signature nucleotides unique to each genomic group. In addition, signature nucleotides common to all isolates of *B. burgdorferi* (i.e. species-specific) were observed. Using *Borrelia* 16S rRNA sequences, probes were constructed that serve to distinguish *B. burgdorferi* from other *Borrelia* species and to distinguish between the genomic groups of *B. burgdorferi*. Other groups have developed *B. burgdorferi* species-specific probes by PCR amplification. We chose rRNA as the target molecule since it is present in large quantities within a cell, so rRNA targets can be considered to be naturally highly amplified. In addition, rRNA molecules are highly conserved and presumably are subject to a very low mutation frequency. The specificity of the probes was demonstrated through the use of slot blots with total cellular RNA as the target. This approach allows the reliable identification and genomic typing of *B. burgdorferi* from cultures, typically within 36 hours. (Garon, Dorward, Marconi).

The Molecular Pathogenesis Unit, under the direction of Dr. Witold Cieplak, Jr., has made important new observations on the biology of human pathogens. They have continued to analyze a number of mutant forms of the enzymatically active A subunit of *E. coli* heat-labile enterotoxin and have identified several residues that appear to be essential to the enzymatic activity of the molecule. Most noteworthy are glutamic acids 110 and 112, tryptophan 127, and histidine 44. Conservative substitutions at these positions do not appear to induce detectable gross conformational changes but reduce the ADP-ribosyltransferase activity to almost undetectable levels. Alterations at these positions are currently being introduced into the holotoxin molecule. To facilitate production of mutant holotoxin analogs, they have subcloned the operon for the holotoxin into a phagemid vector that allows direct mutagenesis at these and other positions. A significant and long-standing question concerning the biological activities of *E. coli* heat-labile enterotoxin concerns the role of limited proteolysis in this phenomenon. Previous results from other laboratories indicated that the specific enzymatic activity and toxicity of the holotoxin molecule was markedly potentiated by treatment with small amounts of trypsin. Structural comparisons to cholera toxin indicated that the trypsin-catalyzed cleavage was likely to occur at an arginine residue at position 192 which results in the formation of a heterodimeric A subunit molecule composed of two disulfide-linked fragments termed A1 and A2. In contrast, they have shown that the enzymatic activity of the isolated recombinant A subunit of *E. coli* heat-labile enterotoxin, although dependent on the presence of reducing agents, is not enhanced by limited trypsinolysis under conditions which produce A1 and A2. As a result, they have investigated the biological effects of blocking tryptic cleavage in the holotoxin molecule by creating a holotoxin mutant with arginine 192 replaced with glycine. Surprisingly, this molecule retains toxicity, as judged by cytotonic effects in Chinese hamster ovary cells, that is equivalent to that of the wild-type toxin. This finding indicates that substitution of this residue blocks enzymatic activation *in vitro* but has little effect on *in vivo* function and argues strongly that intact A subunit can enter cells. Clearly, abrogation of tryptic cleavage is not useful in the generation of detoxified mutants of the enterotoxin. In addition, members of this unit have characterized in detail the interaction of *C. jejuni* with polarized epithelial cells (Caco-2) of human colonic origin. Polarized cells are thought to more closely approximate cells that line epithelial surfaces (e.g. intestinal epithelium). *C. jejuni*, like some other enteroinvasive pathogens is able to translocate across such cell monolayer by a process that involves transient dissolution of tight junctions. Further, the process is also dependent upon bacterial protein synthesis as judged by the inhibitory effect of chloramphenicol on translocation. Using non-polarized epithelial cells they have determined that the new or enhanced synthesis of approximately nine *C. jejuni* proteins, as judged by two-dimensional gel electrophoresis, is closely linked to the internalization process. In addition, the synthesis of several other proteins appears to be "down regulated" during internalization. Antisera have been prepared in rabbits against *C. jejuni* cultivated in the presence or absence of epithelial cells. These antisera exhibit differential reactivities with proteins in extracts from *C. jejuni* cultured in the presence of epithelial cells. The antiserum against the co-cultivated *C. jejuni* is capable of inhibiting internalization significantly (two orders of magnitude). The antisera have been used to differentially screen a genomic expression library of *C. jejuni*, and several recombinant clones have been identified that produce products recognized by one but not both of the antisera. (Cieplak, Konkel)

A major research goal of the Laboratory of Vectors and Pathogens is to characterize in detail those genes and gene products that are important in bacterial pathogenesis. This knowledge will then be exploited for the development of improved diagnostics and safe and effective new-generation vaccines. An additional goal is to understand the mechanisms used by arthropod vectors to maintain and deliver virulent human pathogens.

Drs. Willy Burgdorfer and John Munoz worked as scientist emeritus members of the laboratory during the year, providing valuable service and support.

Guest Researchers in the Laboratory of Vectors and Pathogens have included Dr. Stanley Falkow (Stanford University School of Medicine), Dr. Lucy Tompkins (Stanford University Hospital), Dr. Willard Granath (Division of Biological Sciences, University of Montana), Dr. William Whitmire (Lyme Borreliosis Foundation Fellow).

Joining the Laboratory as an IRTA Fellow during the year was Dr. Joseph Hinnebusch (University of Texas, San Antonio). Completing an IRTA Fellowship was Dr. Kenneth Gage. Joining the Laboratory as Staff Fellows were Dr. William Whitmire, and Dr. Pamela Small (Middlebury College, Vermont). Joining the Laboratory in our Visiting Associateship Program was Dr. Christopher Grant (Stanford University).

Visitors/Collaborators who spent varying amounts of time interacting with members of the LVP Scientific Staff included:

Name	Affiliation
Dr. Harry Malech	NIAID
Dr. Helene Rosenberg	NIAID
Dr. Lisa Pascopella	Albert Einstein University
Dr. Pamela Small	Middlebury College, Vermont
Dr. Patrick Cleary	University of Minnesota
Dr. Richard Friedman	University of Arizona
Dr. Craig Bloch	University of Michigan
Mr. Steve Porcella	University of Montana

Dr. Claude F. Garon

Served as Chairman, State Board of Governors, Center of Excellence in Biotechnology, Montana Science and Technology Alliance

Served as Faculty Affiliate - Division of Biological Sciences, University of Montana

Served on Internal Advisory Committee, Electron Microscopy Facility, University of Montana

Served as Chairman, Montana Governor's Focus Group on Biotechnology, Montana State Department of Commerce

Served on Ph.D. Dissertation Committee, University of Texas Health Science Center at San Antonio, Texas.

Served as Program Chairman, Biochemistry/Medicinal Chemistry Section, 47th Northwest Regional Meeting of the American Chemical Society

Served as Chairman, Research Track, Lyme Disease 1992 State of the Art Conference, Stamford, Connecticut

Served as Chairman, Session on Genome Structure, Molecular Biology of Spirochetes, Annecy, France

Invited Speaker

Department of Microbiology and Immunology, West Virginia University School of Medicine, Morgantown, West Virginia

Department of Microbiology, University of Texas Health Science Center, San Antonio, Texas

Molecular Biology of Spirochetes Conference, Annecy, France

Division of Biological Sciences, University of Montana

Montana Science and Technology Alliance Entrepreneurship Forum Luncheon Speaker, Big Sky, Montana

Reviewed Manuscripts

Journal of Clinical Microbiology

Dr. Tom G. Schwan

Invited Speaker

Annual Meeting of the Northwest Mosquito Vector Control Association, Missoula, Montana

University of Montana, Missoula, Montana

Molecular Biology of Spirochetes, Annecy, France

Tufts, New England Medical Center, Boston, Massachusetts

Annual Meeting of the Entomological Society of America, Reno, Nevada

Annual District Rotary Meeting, Dillon, Montana

Vancouver General and Children's Hospital, Vancouver, British Columbia, Canada

Lyme Borreliosis Foundation Meeting, Stanford, Connecticut

Veterinary Research Laboratory, Montana State University, Bozeman, Montana

Gundersen Clinic, LaCrosse, Wisconsin

First International Conference on Tick-borne Disease, St. Paul, Minnesota

Committee/Board Appointments

Executive Council, American Committee of Medical Entomology
National Scientific Advisor, American Lyme Disease Foundation
Editorial Board Journal of Clinical Microbiology
Organization Committee, First International Conference on Tick-borne Diseases

Reviewed Manuscripts

American Journal of Tropical Medicine and Hygiene
Journal of Clinical Microbiology
Journal of Immunology
Journal of Medical Entomology
Infection and Immunity
Proceedings of the International Academy of Science

Dr. Witold Cieplak Jr.

Invited Speaker

University of Arizona, College of Veterinary Sciences, Tucson, Arizona

Reviewed Manuscripts

Infection and Immunity (Editorial Board Member)
Journal of Immunology

Dr. Willy Burgdorfer

Invited to present guest lecture "Ein Jahrzehnt nach der Entdeckung des Erregers der Lyme Borreliose" at the 45th Van Swieten Congress of the Austrian Physicians, Vienna, Austria.

Invited to present guest lecture on Lyme Borreliosis at the Department of Dermatology, Bern, Switzerland.

Participated in ceremonies to receive Honorary Doctor Medicine Degree at the University of Marseille, France.

Invited to present lecture "Status of Lyme disease as a global illness," Medical Faculty, University of Marseille, Marseille, France.

Invited to present lecture on "Epidemiology of Lyme disease: at the NIAID-sponsored Conference on Emerging Microbes and Microbial Diseases, Washington DC.

Presented Course on Tick-borne Diseases in the Northwest to 55 Forest Service Employees, Missoula, Montana.

Presented lecture on "Global Perspective of Lyme Disease: at the Lyme Disease 1992 State of the Art Conference," Stanford, Connecticut.

Invited to serve as Chairman at V International Conference on Lyme Borreliosis, Arlington, Virginia.

Invited to serve as Moderator and Speaker on "Lyme Borreliosis: Ten Years After Discovery of the Etiologic Agent, *Borrelia burgdorferi*"at the Lyme Disease 1992 Conference, LaCrosse, Wisconsin.

Served as instructor at 42nd Acarology Summer Program, Ohio State University, Columbus, Ohio.

Invited to present lecture "Lyme disease: a global perspective" at Ohio Department of Health, and Columbus Health Department, Columbus, Ohio.

Continued serving as Co-Editor of the book "Aspects of Lyme Borreliosis," 29 chapters, Springer Verlag (in press).

Dr. David W. Dorward

Invited Speaker

Ribi ImmunoChem Research, Incorporated, Hamilton, Montana

Poster Session - Montana BioTechnology Conference, Missoula, Montana

American Society for Microbiology General Meeting, New Orleans, Louisiana

Poster Session - International Conference on Lyme Borreliosis, Arlington, Virginia

American Chemical Society, Northwest Branch, Missoula, Montana

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00480-07 LVP

PERIOD COVERED
October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Sr Staff Fellow	LVP, NIAID
OTHERS:	K. L. Gage	IRTA Fellow	LVP, NIAID
	J. Hinnebusch	IRTA Fellow	LVP, NIAID
	R. H. Karstens	Bio Lab Tech (Micro)	LVP, NIAID
	W. Burgdorfer	Scientist Emeritus	LVP, NIAID
	E. Kamanga-Solla	Visiting Scientist	LMSF, NIAID

COOPERATING UNITS *(If any)*
Microbial Diseases Laboratory, California Department of Health, Berkeley (Jane Wong); University of Texas Health Science Center (Alan Barbour)

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Arthropod-borne Diseases

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The objectives of this project are to (1) use molecular and classical approaches to investigate pathogen-arthropod interactions of vector-borne diseases of human importance in the United States, (2) examine basic biological questions concerning the behavior of ticks and fleas that influence their ability to transmit infectious agents, and (3) to develop rapid and definitive diagnostic assays to detect and identify bacterial pathogens in their arthropod vector. Several accomplishments were made concerning our third objective during the last year. First, we developed a monoclonal antibody (H9826) that bound to the flagellar protein of *Borrelia hermsii*, an agent of tick-borne relapsing fever, but not to flagellin of any of the other species tested, which included *B. parkeri*, *B. turicatae*, *B. coriaceae*, *B. anserina*, *B. burgdorferi*, and *Leptospira interrogans*. This antibody bound efficiently to *B. hermsii* in an indirect immunofluorescence assay and was used to rapidly detect and identify this spirochete in the peripheral blood of experimentally infected mice and in the central ganglia of *Ornithodoros hermsi* ticks. We have recently used this antibody to rapidly identify *B. hermsii* in the blood of human patients acutely ill with relapsing fever. Second, we adapted the polymerase chain reaction (PCR) for identifying rickettsial infections in ticks. Our assay amplified a 500 base pair DNA fragment from the gene encoding the rOmp B protein of *Rickettsia rickettsii*. The assay amplified fragments of the predicted size from all spotted fever group rickettsiae tested but not from any typhus group rickettsiae. We specifically amplified rickettsial DNA from the hemolymph, saliva, and triturated leg tissues from live, partially fed ticks. Samples of infected ticks preserved in 70% EtOH were also suitable for amplification by PCR. Also, we have successfully amplified *Yersinia pestis* DNA in experimentally infected fleas, *Xenopsylla cheopis*. This assay will provide a long overdue rapid method for detecting plague bacteria in mammalian hosts and vector tissues.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864 939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00488-06 LVP

PERIOD COVERED
October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ultrastructural Analysis of Antigenic Determinants in Pathogens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Chief	LVP, NIAID
OTHERS:	S. F. Hayes	Bio Lab Tech (Micro)	LVP, NIAID
	W. Burgdorfer	Scientist Emeritus	LVP, NIAID
	D. W. Dorward	Sr Staff Fellow	LVP, NIAID
	L. L. Lubke	Biologist	LVP, NIAID
	T. G. Schwan	Sr Staff Fellow	LVP, NIAID
	W. M. Whitmire	Sr Staff Fellow	LVP, NIAID

COOPERATING UNITS *(if any)*
LPVD, RML, NIAID; LMSF, RML, NIAID; LICP, RML, NIAID; Dept. of Medical Microbiology, Stanford Univ. School of Medicine

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Structural Pathobiology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	2.2	2.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

This project serves to consolidate and to expand our efforts at identifying, localizing, and characterizing genes and gene products which are important in host pathogen relationships.

Recent experiments have shown that nuclease-protected DNAs are exported from *Borrelia burgdorferi* cells in association with membrane vesicles. Indirect evidence suggests that these vesicles may be produced by spirochetes *in vivo*, providing a sustained antigenic challenge to hosts that maintain a limited population of spirochetes. To determine whether *B. burgdorferi* vesicles occur in experimentally infected mice, polyclonal rabbit sera were generated against vesicles and against a prominent, vesicle-associated protein and with an apparent mass of 83 kDa. Using these reagents, we developed an immune electron microscopic assay for first capturing and then identifying extracellular *B. burgdorferi* antigens. We then extended the study to include antigen detections in *Ixodes* ticks and in mouse, dog, and human samples. This approach has proven effective for demonstrating *B. burgdorferi* infections, and should enable further ultrastructural examination of the effects of these bioproducts on hosts.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00492-06 LVP

PERIOD COVERED
October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Basis for Infection by *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Sr Staff Fellow	LVP, NIAID
OTHERS:	J. Hinnebusch	IRTA Fellow	LVP, NIAID
	R. H. Karstens	Bio Lab Tech (Micro)	LVP, NIAID
	M. E. Schrumpf	Bio Lab Tech (Micro)	LVP, NIAID
	P. A. Rosa	Sr Staff Fellow	LMSF, NIAID

COOPERATING UNITS *(if any)*
Microbial Diseases Laboratory, California Department of Health, Berkeley (Jane Wong); University of California, Berkeley (Robert Lane); Tufts-New England Medical Center, Boston (Mark Klempner).

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Arthropod-borne Diseases

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	0.9	1.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

The objectives of this project are to (1) use recombinant DNA techniques to express specific antigens of *Borrelia burgdorferi* to improve the serodiagnosis of Lyme disease, (2) characterize at the molecular level, isolates of the Lyme disease spirochete from a wide range of biological and geographical sources, and (3) identify molecular determinants of the spirochete that are important for infectivity in mammalian and tick hosts.

As part of our continued interest in the 39 kilodalton (P39) protein of *B. burgdorferi* as a diagnostic antigen, analyses were completed with other species of bacteria. Five serovars of *Leptospira interrogans*, *L. biflexa*, *Leptonema illini*, and *Rickettsia rickettsii* were examined and found not to contain this antigen. The specificity of this antigen and its reactivity with human Lyme disease sera should exclude the possibility of false-positive serum samples from patients having had either leptospirosis or Rocky Mountain spotted fever, as well as tick-borne relapsing fever and syphilis, as we discussed in previous reports.

We have examined 31 isolates of *B. burgdorferi* from pools of *Ixodes pacificus* ticks collected from numerous regions spanning most of the state of California where this tick is found. All isolates were identified as *B. burgdorferi* by their reactivities with monoclonal antibodies to outer surface proteins A and B, flagellin, and the 39 kDa (P39) protein. By polymerase chain reaction (PCR) assays, all of the isolates reacted as North American-type *B. burgdorferi*. Plasmid profiles were quite diverse and only 10 of 27 isolates examined contained the small 8 kilobase supercoiled plasmid we described previously. One isolate was made from ticks collected at Wawona Campground in Yosemite National Park, documenting the occurrence of the Lyme spirochete in an area of extreme human recreational use.

We have also developed a rapid technique to identify small supercoiled plasmids by photonicking the supercoiled molecules with UV light and ethidium bromide in agarose gels. This allows us to then identify newly-formed open circular molecules that migrate slower than supercoiled molecules in the gel.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 AI 00552-04 LVP

PERIOD COVERED
October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunopathobiology of Bacterial Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Cieplak, Jr.	Sr Staff Fellow	LVP, NIAID
OTHERS:	C. C. R. Grant	Visiting Associate	LVP, NIAID
	R. J. Messer	Microbiologist	LVP, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Molecular Pathogenesis Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Our efforts to elucidate the structure-function relationships associated with the cholera toxin family of enterotoxins have continued. These toxins, which act by ADP-ribosylating eukaryotic G proteins, are considered to be important antigens for potential inclusion into vaccines for diarrheal disease mediated by organisms such as *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. These toxins also possess potent mucosal adjuvant properties. Accordingly, we are attempting to identify the primary structural requirements associated with toxicity, antigenicity/immunogenicity, and adjuvanticity.

We have successfully expressed the genes for both the entire operon and the gene for the enzymatically active A subunit of *E. coli* heat-labile enterotoxin (LT) in vectors that permit single stranded mutagenesis procedures. Site-specific mutants of codons encoding various amino acids have allowed us to identify residues in the A subunit that appear to be critical to the maintenance of enzymatic activity. Such residues include glutamic acid 110, glutamic acid 112, tryptophan 124, and histidine 44. Mutations within the amino-terminal region of the A subunit also reduce enzymatic activity, but impart gross conformational changes and as such do not appear to be suitable targets for the production of inactivated vaccine candidates. More recently, similar mutations have been introduced into the operon encoding the entire toxin to verify that elimination of enzymatic activity also abrogates toxicity. In addition, we have determined, using site-directed mutagenesis that trypsin-like proteolytic cleavage of LT is not requisite to toxicity, a fact that alters our perception of the intracellular processing and fate of the toxin.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-884-038

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI-00553-04 LVP

PERIOD COVERED
October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology and Immunology of Pathogenic *Campylobacter* spp.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Cieplak, Jr.	Sr Staff Fellow	LVP, NIAID
OTHERS:	M. E. Konkel	IRTA Fellow	LVP, NIAID
	D. J. Meade	Microbiologist	LVP, NIAID

COOPERATING UNITS *(If any)*
Stanford University Medical School (Tompkins); University of Arizona College of Veterinary Sciences (Joens)

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Molecular Pathogenesis Unit

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.5	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

We have continued to investigate potential pathogenic mechanisms associated with enteritis caused by the common pathogen *Campylobacter jejuni*. We have focused our attention on the ability of this organism to bind to and enter epithelial cells in vitro and have described the characteristics of this interaction. During the past year, we have demonstrated that *C. jejuni* can effectively translocate across polarized epithelial cell monolayers in a fashion similar to that of other enteroinvasive pathogens such as *Salmonella typhimurium* and that the translocation is dependent upon active bacterial protein synthesis. High resolution electrophoretic techniques have been used to characterize the number and nature of proteins that are synthesized by *C. jejuni* during interaction with epithelial cells. Other evidence indicates that these proteins are directly involved in facilitating the internalization of *C. jejuni* by epithelial cells. Antisera have been prepared which differentially react with *C. jejuni* cultured in the presence and absence of cells. These antisera have been used to identify recombinant clones in genomic libraries that produce novel *C. jejuni* proteins that are preferentially associated with bacteria cultured in the presence of epithelial cells. These latter studies should permit identification of essential *C. jejuni* virulence determinants.

PHS 6040 (Rev 1/84)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00554-04 LVP

PERIOD COVERED
October 1, 1991, to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Characterization of Microbial Genes and Nucleic Acid Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Chief	LVP, NIAID
OTHERS:	R. T. Marconi	IRTA Fellow	LVP, NIAID
	D. W. Dorward	Sr Staff Fellow	LVP, NIAID
	L. L. Lubke	Biologist	LVP, NIAID
	D. S. Samuels	IRTA Fellow	LVP, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Vectors and Pathogens

SECTION
Structural Pathobiology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.3	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided..)*

Lyme borreliosis is now the most common arthropod-borne disease in the United States. *Borrelia burgdorferi*, the causative agent, has been isolated from humans, mammals, birds and arthropods and is cultivable in the laboratory. Careful structural analysis of the DNA content of several early passage isolates has revealed, in addition to a 1,000 kb linear genome, a unique mixture of terminally cross-linked linear and covalently-closed, circular DNA molecules ranging in size from 2 to 50 kilobases in length. Often DNA patterns appear to differ among isolates and to vary during laboratory passage.

While all of the linear species were shown to rapidly reanneal to linear duplexes after alkaline denaturation, single-stranded circular molecules measuring twice the length of the linears could be produced by treatment with methyl mercury, glyoxal and urea prior to mounting for electron microscopy. This rapid reannealing mediated by the terminal sequences of the molecule was used in a unique ion exchange column chromatography system to purify terminal, restriction fragments from any which originate internally. Purified terminal fragments could then be compared and characterized further by conventional methods.

The use of rRNA sequences as chronometers of evolutionary divergence is now widespread and its advantage over classical taxonomic approaches is well documented. To date, over 500 small subunit rRNA sequences have been determined and used for this purpose. We have determined in near entirety, the 16S sequences of several borrelial species and isolates. The phylogenetic relationships among the isolates were determined through calculations of sequence similarity and Knuc values and the data presented as an unrooted phylogenetic tree. In addition, rDNA restriction patterns were determined using Southern blot analyses, and the data compared with published genomic grouping schemes.

The objective of this project, therefore, is to define the genetic capacity of *B. burgdorferi* and other vector-borne pathogens in sufficient detail to begin the process of mapping those genes and gene products which may be important in pathogenesis.

PHS 6040 (Rev 1/84)

U.S. GOVERNMENT PRINTING OFFICE 1991 0 864 939

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1992 Annual Report
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1991 to September 30, 1992

Introduction

It was decided to move responsibility for many administrative functions from the Operations Branch to the Administrative Management Branch, DIR, NIAID, similar to the organization on the main campus. To facilitate this change, a RML Business Management Section in the Administrative Management Branch, DIR was organized at RML with Dr. Tonu Wali as the Section Chief. Eight staff members in the Office of the Chief, RMOB were transferred into the new RML Business Management Section. This section is responsible for the planning, directing, and coordinating of the administrative management functions for the RML, including personnel management, financial management, accounting management, property management, space management, data management, office/lab automation, procurement, and other administrative functions necessary to ensure efficient and effective implementation of the overall Division of Intramural Research and RML program objectives. The Operations Branch continues to provide support in the following areas: library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of the power plant, and full maintenance and minor laboratory renovations in every area except electronics.

We have continued to upgrade the facilities in the Animal Care Section. An additional animal procedure room was established and equipped with appropriate furnishings and a scavenging system for ether anesthesia. Continued progress was made in replacing old galvanized steel equipment and replacing old worn out caging. The use of microisolator caging and clean air work stations continued to increase. Water acidification systems were ordered and installed in Bldgs. 12, 13, and HD-3. The mink facilities underwent extensive refurbishing including new caging, nest boxes, feeding system and a large barn enclosure for normal animals.

The Maintenance Section renovated a suite of rooms in Bldg. 2 to provide modern laboratory space for the new Cell Biology Section in the Laboratory of Vectors and Pathogens. In addition, the Maintenance Section has performed other major and minor maintenance and renovation projects for the Animal Care Section, LMSF, LPVD, LICP and LVP.

Operations Branch personnel have assisted in providing host support for three meetings held at RML in FY 92. The Edna McConnell Clark Foundation Workshop on *In Vitro* Chlamydial Neutralization was held during the week of July 20 - 21 by LICP. LMSF sponsored a meeting entitled "The Interplay of Microbes and Their Hosts" on July 30 - August 1. The tenth Sesqui-Annual meeting of the American Society for Rickettsiology and Rickettsial Diseases was held on September 17 - 19.

General Overview of the Responsibilities of Operations Branch

The Chief of the Branch is responsible for labor management work and administrating the technical aspects of the contracts for Security, Custodial, operation of the Boiler Room and the Glassware Unit with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Boiler Room operation provides heat, steam, compressed air, vacuum, and emergency power to the entire laboratory complex. Glassware is cleaned and sterilized in the Glassware Unit for reuse in the laboratories.

The Maintenance Department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning and refrigeration, including ultra-low temperature boxes. With exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.

Most of the biological media used in research laboratories is prepared in a media kitchen by a technician.

The Graphic Arts Department provides full professional services to the laboratory staffs with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Care Section raises 2 strains of guinea pigs, 21 strains of mice, 7 strains of hamsters, a colony of grasshopper mice, a colony of peromyscus and a colony of microtus. They are raising 45,000 animals annually for research from an average pool of 3,000 breeding stock (approximate figures). An additional 3,000 to 4,000 animals are purchased annually from outside sources, including mink, rabbits, rats, mice, hamsters and guinea pigs. After rearing, care is provided for these animals while they are under experiment. The most recent survey indicates an average daily holding of 14,000 small animals. An experimental colony of 39 cynomolgus macaques, as well as 2 horses, 2 cattle and 2 sheep are also maintained in the animal facilities.

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